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<b>(54) Title:</b> NOVEL MOLECULES			
<b>(57) Abstract</b> <p>The present invention relates generally to novel molecules and more particularly novel proteinaceous molecules involved in or associated with regulation of cell activities and/or viability. The present invention is particularly directed to novel serine proteinases and a novel kinase and to derivatives, agonists and antagonists thereof. In one embodiment, the present invention provides a novel serine proteinase, referred to herein as "HELA2" or "testisin", which has roles in spermatogenesis, in suppressing testicular cancer and as a marker for cancers.</p>			

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## NOVEL MOLECULES

### FIELD OF THE INVENTION

5 The present invention related generally to novel molecules and more particularly novel proteinaceous molecules involved in or associated with regulation of cell activities and/or viability. The present invention is particularly directed to novel serine proteinases and a novel kinase and to derivatives, agonists and antagonists thereof. In one embodiment, the present invention provides a novel serine proteinase, referred to herein as "HELA2" or "testisin", which  
10 has roles in spermatogenesis, in suppressing testicular cancer and as a marker for cancers.

### BACKGROUND OF THE INVENTION

The rapidly increasing sophistication of recombinant DNA technology is greatly facilitating  
15 research and development in the medical and allied health fields. This is particularly the case in the area of cell regulation leading to a greater understanding of the events leading to or involved in cancer, development of acquired immunodeficiency disease syndrome (AIDS), neurological disorders, heart disease, tissue graft rejection and infertility amongst many other conditions.

20 Two particularly important classes of molecules are the proteinases and kinases.

Proteinases play important roles in a number of physiological and pathological processes such as proteolytic cascades involved in blood coagulation, fibrinolysis and complement activation as well as cleavage of growth factors, hormones and receptors, the release of bioactive molecules  
25 and processes involving cell proliferation and development, inflammation, tumour growth and metastasis. Of particular significance are the cellular proteinases, or those proteinases synthesized in cells and tissues which serve to activate or deactivate proteins responsible for performing specific functions. These proteinases may be found outside the cell, within the cell or may be present on the cell surface.

30

Serine proteinases are particularly important. These proteinases are characterised by a

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mechanism involving serine, histidine and aspartate amino acids in the serine proteinase active site. Members of the serine proteinase family which play important roles in a range of cellular functions and which have demonstrated causative roles in human diseases include tissue-type plasminogen activator and thrombin (thrombosis and blood clotting), urokinase-type plasminogen  
5 activator (cancer and metastasis), trypsin and elastase (emphysema and liver disease) and angiotensin converting enzyme (hypertension).

A serine proteinase is also implicated in TNF $\alpha$  degradation and soluble TNF-receptor (p75) release by THP1 cells (Vey *et al. Eur. J. Imm.* 26, 2404-2409, 1996). Serine proteinases have  
10 been implicated in the activation of macrophages (Nakabo *et al. J. Leukocyte Biol.* 60, 328-336, 1996), in nuclear laminin degradation in apoptosis (McConkey *et al. J. Biol. Chem.*, 271, 22398-22406, 1996), in prostaglandin-E2 induced release of soluble TNF receptor shedding (Choi *et al. Cellular Immunology* 170, 178-184, 1996), in PAF synthesis (Bussolino *et al. Eur. J. Immunol.* 24, 3131-3139, 1994), and in the proteolysis of I $\kappa$ B, a regulatory molecule important  
15 in signal transduction and apoptosis. Release of serine proteinases known as granzymes is central to CTL killing and many of the substrates cleaved by granzymes are also cleaved by cellular proteinases (for example, IL-1 $\beta$  is a substrate for Granzyme B as well as the cysteine proteinase, interleukin 1 $\beta$ -converting enzyme (ICE)). Granzyme A, a serine proteinase with Arg-amidolytic activity, has been reported to induce the production of IL-6 and IL-8 in lung fibroblasts (Sower  
20 *et al. Cellular Immunology* 171, 159-163, 1996) and cleaves IL-1 $\beta$  to a 17kD mature form that is biologically active.

Kinases are a large group of molecules, many of which regulate the response of cells to external stimuli. These molecules regulate proliferation and differentiation in eukaryotic cells frequently  
25 via signal transduction pathways.

The identification of new serine proteinases and kinases permits the development of a range of derivatives, agonists and antagonists at the nucleic acid and protein levels which in turn have applications in the treatment and diagnosis of a range of conditions such as cancer, inflammation,  
30 neurological disorders amongst many other conditions including conditions which initiate or promote apoptosis such as viral infection, old age and drug abuse. One particularly useful serine

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proteinase HELA2 (testisin) identified in accordance with the present invention is involved in spermatogenesis, testicular cancer and as a marker for cancer.

## SUMMARY OF THE INVENTION

5

Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined at the end of the subject specification.

Throughout this specification and the claims which follow, unless the context requires otherwise, 10 the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

One aspect of the present invention provides a novel molecule in isolated form involved in or 15 associated with regulation of cell activity and/or viability.

Another aspect of the present invention contemplates an isolated proteinaceous molecule involved in or associated with regulation of cell activity and/or viability comprising a sequence of amino acids encoded by a nucleotide sequence, at least a portion of which, is capable of being 20 amplified by polymerase chain reaction (PCR) using the following primers:

5' ACAGAATTCTGGGTIGTGTACIGCIGCICAYTG3' [SEQ ID NO:1]; and

5' ACAGAATTCA XIGGICCCIC/GT/AXTCICCC3' [SEQ ID NO:2];

25

or a complementary form of said primers.

The proteinaceous molecule of the present invention may be a serine proteinase or a kinase.

30 Yet another aspect of the present invention is directed to an isolated serine proteinase comprising the amino acid sequence substantially set forth in SEQ ID NO:4 or an amino acid sequence

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having at least about 50% similarity to all or part thereof. This serine proteinase is referred to herein as a short isoform (S) of "HELA2" or "testisin".

Still another aspect of the present invention relates to an isolated serine proteinase comprising  
5 the amino acid sequence substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least about 50% similarity to all or part thereof. This serine proteinase is referred to herein as a long isoform (L) of HELA2 (testisin).

Still yet another aspect of the present invention provides an isolated serine proteinase comprising  
10 an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least about 50% similarity to all or part thereof. This serine proteinase is referred to herein as "ATC2".

Even yet another aspect of the present invention is directed to a serine proteinase in isolated form  
15 comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 50% similarity to all or part thereof or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:3 under low stringency conditions at 42°C.

20 Another aspect of the present invention relates to a serine proteinase in isolated form comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:5 or a nucleotide sequence having at least 50% similarity to all or part thereof or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:5 under low stringency conditions at 42°C.

25

Still another aspect of the present invention provides a serine proteinase in isolated form comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:7 or a nucleotide sequence having at least 50% similarity to all or part thereof or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:7 under  
30 low stringency conditions at 42°C.

Another embodiment of the present invention is directed to a kinase in isolated form comprising an amino acid sequence substantially as set forth in SEQ ID NO:10 or having 50% amino acid similarity to all or part thereof. This kinase is referred to herein as "BCON3".

- 5 In a related embodiment, the kinase comprises an amino acid sequence encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:9 or a nucleotide sequence having at least 50% similarity to all or part of the nucleotide sequence set forth in SEQ ID NO:9 or a nucleotide sequence capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:9 under low stringency conditions at 42°C.

10

The present invention further provides an isolated nucleic acid molecule encoding a polypeptide wherein at least a portion of said nucleic acid molecule is capable of being amplified by polymerase chain reaction (PCR) using the following primers:

- 15 5' ACAGAATTCTGGGTIGTACIGCIGCICAYTG3' [SEQ ID NO:1]; and

5'ACAGAATTCAXIGGCCICCCIC/GT/AXTCICCC3' [SEQ ID NO:2];

or a complementary form of said primers.

20

The present invention also provides an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:3 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:3 under low stringency conditions at 42°C.

25

Another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:5 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:5 under low stringency conditions at 42°C.

30

Still another aspect of the present invention is directed to an isolated nucleic acid molecule

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comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:7 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:7 under low stringency conditions at 42°C.

- 5 Even still another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:9 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:9 under low stringency conditions at 42°C.
- 10 Another aspect of the present invention provides an isolated serine proteinase encoded by a gene proximal to a cluster of genes on a mammalian chromosome.

More particularly, this aspect of the present invention is directed to a serine proteinase encoded by a gene proximal to a cluster of genes on human chromosome 16p13.3 or its equivalent in a  
15 non-human species.

- Still more particularly, the serine proteinase is encoded by a gene comprising a nucleotide sequence substantially as set forth in SEQ ID NO:3 or 5 or 28 or 29 or 30 or a nucleotide sequence having at least 50% similarity to any one thereof or a nucleotide sequence capable of  
20 hybridizing to any one of SEQ ID NO:3 or 5 or 28 or 29 or 30 under low stringency conditions at 42°C or a nucleotide sequence encoding a serine proteinase having an amino acid sequence substantially as set forth in SEQ ID NO:4 or 6 or an amino acid sequence having at least about 50% similarity to SEQ ID NO:4 or 6.

25



## BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** is a representation showing (A) schematic and (B) hydrophobicity plot of the HELA2 amino acid sequence.

5

**Figure 2** is a diagrammatic representation showing: (A) the amino acid sequence of HELA2 (testisin). The putative signal sequence, light chain, heavy chain and transmembrane domains are as indicated, the catalytic amino acids, His, Asp and Ser are as designated; insertion of Tyr-Ser (YS) 4 amino acids after the catalytic His is found in the long isoform of testisin and is due to  
10 alternative mRNA splicing; (B) *in vitro* transcription/translation of HELA2 (testisin) showing the protein product.

**Figure 3** is a diagrammatic representation of plasmid constructs pBluescriptHELA2(S) and pBluescriptHELA2(L) containing full length cDNAs for testisin (short isoform (S)) and testisin  
15 (long isoform (L)), respectively.

**Figure 4** is a diagrammatic representation of plasmid constructs pQET(20-295)N and pQET(20-295)C, wherein the hydrophobic residues of testisin were removed and the remaining sequences cloned into pQE prokaryotic expression plasmids; plasmids pGEX-1 (90-279) comprising a  
20 carboxy terminal part of testisin fused to glutathione-S-transferase.

**Figure 5** is a photographic representation of: (A) silver stained gel showing purification of recombinant HELA2 (testisin) from *E. coli*. The purified HELA2 (testisin) is indicated by the arrow in the eluate fractions. Some HELA2 (testisin) is also found in the wash fractions as the  
25 affinity matrix was not used in excess. His-N21 is one clone containing the amino-terminal His tag, and clones His-C21, His-C22 and His-C23 are three different clones with the carboxy-terminal His tag. (B) Western blot of native and denatured recombinant HELA2 (testisin) probed with Clontech anti-His tag-antibody. The 32kD band shown by the arrow is HELA2 (testisin). HELA2 (testisin) is not detected in the denatured samples as it appears that  
30 denaturation with urea destroys the His epitope recognised by the monoclonal antibody.

**Figure 6** is a representation of the amino acid sequence of HELA2 (testisin) showing the regions of the molecule selected for generation of peptide antigens.

**Figure 7** is a photographic representation of a Western blot of GST-HELA2 (testisin) fusion protein purified by affinity chromatography.

**Figure 8** is a diagrammatic representation of eukaryotic expression constructs, pcDNA3-Test(S-C), pcDNA3-Test(L-C) and pcDNA3-Test(1-297)L-C.

**Figure 9** is a diagrammatic representation showing a histogram of the signal intensity from a Clontech Master RNA blot of the tissue distribution of HELA2 (testisin) in RNA from 50 different normal tissues. (A) Probed with HELA2 (testisin) specific probe; (B) Probed with BCON3 specific probe which is ubiquitously expressed. The 8 tissues on the right hand side of the diagram are the control (negative) samples.

15

**Figure 10** is a photographic representation of a multiple normal tissue Northern blot (Clontech) probed with: (A) HELA2 (testisin) specific probe and (b) BCON3 specific probe.

**Figure 11** is a photographic representation of agarose gel of PCR products generated by amplification of HELA2 (testisin) cDNA in prevasectomised and post-vasectomised ejaculate specimens. The HELA2 (testisin) PCR product is 464bp and the  $\beta$ 2-macroglobulin product is 250 bp.

**Figure 12** is a photographic representation of *in situ* hybridization of rat testis showing the localisation of HELA2 (testisin) mRNA to the germ cells of the testis.

**Figure 13** is a representation showing: (A) spread of normal metaphase chromosomes showing bright dots where HELA2 (testisin) is expressed at 16p13.3; (B) Diagrammatic representation of chromosome 16p13.3 showing location of HELA (testisin) and relationship to other disease causing genes.

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**Figure 14** is: (A) a photographic representation of northern blot analysis of HELA2 (testisin) mRNA showing signals in normal testis of 4 patients and absence of signal in the tumours of these patients; (B) a photographic representation of the localisation of HELA2 (testisin) protein in a human germ cell tumour section assessed by immunohistochemical staining using anti-HEL A2 (testisin) peptide antibodies. Staining is only detected in the normal (N) tissue and not present in the tumour (T) tissue.

**Figure 15** is a diagrammatic representation of the genomic map of HELA2 (testisin) showing experimentally determined intron/exon boundaries and relative sizes of the introns (marked with a letter) and exons (marked with a roman numeral).

**Figure 16** is a representation of HELA2 (testisin). Nucleotides in introns are in lowercase and exons in uppercase. The putative transcription start site is marked by +1.

**Figure 17** is a representation of the DNA sequence of Intron C and flanking exons showing where alternative mRNA splicing occurs to generate the two isoforms of HELA2 (testisin).

**Figure 18** is a representation of: (A) the cDNA sequence of the mouse homologue of HELA2 (testisin). Catalytic residues are indicated by circles and cysteines likely involved in disulfide bonding are indicated by squares; (B) Hydrophobicity plot of HELA2 (testisin) amino acid sequence.

**Figure 19** is a diagrammatic representation of chromosome 16p13.3 showing the serine proteinase gene cluster which includes HELA2 (testisin). Lines represent cosmids containing the respective serine proteinase genes.

**Figure 20A** is a representation of: (A) the cDNA sequence of SP001LA (SEQ ID NO:28). Catalytic residues are indicated by circles and cysteins likely involved in disulfide bonding are indicated by squares; (B) hydrophobicity plots of SP001LA amino acid sequence.

**Figure 20B** is a representation of: (A) the cDNA sequence of SP002LA (SEQ ID NO:29).

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Catalytic residues are indicated by circles and cysteines likely involved in disulfide bonding are indicated by squares. (B) Hydrophobicity plot of SP002LA amino acid sequence.

**Figure 20C** is a representation of: (A) the cDNA sequence of SP003LA (SEQ ID NO:30).  
5 Catalytic residues are indicated by circles and cysteines likely involved in disulfide bonding are indicated by squares. (B) Hydrophobicity plot of SP003LA amino acid sequence.

**Figure 21** is a photographic representation of *in vitro* transcription/translation of BCON3 showing the protein products.

A summary of the SEQ ID NOs used throughout the specification is presented in Table 1.

TABLE 1

	SEQ ID NO	DESCRIPTION
5	1	* PCR primer sequence
	2	* PCR primer sequence
	3	Nucleotide sequence of short form of HELA2
	4	Amino acid sequence of short form of HELA2
10	5	Nucleotide sequence of long form of HELA2
	6	Amino acid sequence of long form of HELA2
	7	Nucleotide acid sequence of ATC2
	8	Amino acid sequence of ATC2
	9	Nucleotide acid sequence of BCOM3
15	10	Amino acid sequence of BCOM3
	11	Primers used to generate amino terminal tagged protein
	12	Primers used to generate amino terminal tagged protein
	13	Primers used to generated carboxy-linked terminal protein
	14	Primers used to generated carboxy-linked terminal protein
20	15	Peptide antigen T20-33
	16	Peptide antigen T46-63
	17	Peptide antigen T175-190
	18	Forward primer
	19	Reverse primer
25	20	Forward primer
	21	Reverse primer

TABLE 1 (Continued)

5	22	Forward primer
	23	Reverse primer
	24	Serine proteinase activation motif
	25 & 26	Mouse HELA2 cDNA sequence
	27	Human genomic DNA sequence
10	28	Clustered serine proteinase gene SP001LA
	29	Clustered serine proteinase gene SP002LA
	30	Clustered serine proteinase gene SP003LA

\* Abbreviations: X= A or G

Y= C or T

15 I= Inosine.

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A list of single and three letter abbreviations for amino acid residues is presented in Table 2.

TABLE 2

5	Amino Acid	Three-letter Abbreviation	One-letter Symbol
	Alanine	Ala	A
	Arginine	Arg	R
10	Asparagine	Asn	N
	Aspartic acid	Asp	D
	Cysteine	Cys	C
	Glutamine	Gln	Q
	Glutamic acid	Glu	E
15	Glycine	Gly	G
	Histidine	His	H
	Isoleucine	Ile	I
	Leucine	Leu	L
	Lysine	Lys	K
20	Methionine	Met	M
	Phenylalanine	Phe	F
	Proline	Pro	P
	Serine	Ser	S
	Threonine	Thr	T
25	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
	Valine	Val	V
	Any residue	Xaa	X

## DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention is predicated in part on a genetic engineering approach to identify nucleotide sequences encoding serine proteinases or kinases. The genetic engineering approach is based on the use of degenerate primers corresponding to conserved regions of serine proteinases (amino acids flanking His- and Ser- residues) to amplify gene fragments spanning these regions for cDNA, using low stringency reverse transcriptase-polymerase chain reaction (RT-PCR).

- 10 This technique has been successfully used, in accordance with the present invention, to identify serine proteinases and kinases useful in modulating cell activity and viability including modulating spermatogenesis, acting as tumour suppressors and acting as a marker for non-testicular cancers.

Accordingly, one aspect of the present invention provides a novel molecule in isolated form involved in or associated with regulation of cell activity and/or viability.

More particularly, the present invention contemplates a novel serine proteinase in isolated form comprising a sequence of amino acids encoded by a nucleotide sequence, at least a portion of which, is capable of being amplified by polymerase chain reaction (PCR) using the following primers:

5' ACAGAATTCTGGGTIGTACIGCIGCICAYTG3' [SEQ ID NO:1]; and

5' ACAGAATTCAXIGGCCICCCIC/GT/AXTCICCC3' [SEQ ID NO:2];

25

or a complementary form of said primers.

Preferably, X is A or G, Y is C or T and I is inosine.

- 30 In a particularly preferred embodiment, the isolated serine proteinase comprises the amino acid sequence substantially set forth in SEQ ID NO:4 or an amino acid sequence having at least about



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50% similarity to all or part thereof. This serine proteinase is referred to herein as a short isoform of "HELA2" or "HELA2 (testisin)". The terms "HELA2" and "testisin" are used interchangeably throughout the subject specification to refer to the same molecule.

5 In another preferred embodiment, the amino acid sequence of the serine proteinase is substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least about 50% similarity to all or part thereof. This serine proteinase is the long isoform of HELA2 or HELA2 (testisin).

10 Yet another preferred embodiment of the present invention provides an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least about 50% similarity to all or part thereof. This serine proteinase is referred to herein as "ATC2".

Another aspect of the present invention relates to a serine proteinase in isolated form comprising  
15 a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 50% similarity to all or part thereof or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:3 under low stringency conditions at 42°C.

20 Still another aspect of the present invention is directed to a serine proteinase in isolated form comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:5 or a nucleotide sequence having at least 50% similarity to all or part thereof or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:5 under low stringency conditions at 42°C.

25

In another aspect of the present invention, there is provided a serine proteinase in isolated form comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:7 or a nucleotide sequence having at least 50% similarity to all or part thereof or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:7 under  
30 low stringency conditions at 42°C.

Another embodiment of the present invention is directed to a kinase in isolated form comprising an amino acid sequence substantially as set forth in SEQ ID NO:10 or having 50% amino acid similarity to all or part thereof. This kinase is referred to herein as "BCON3".

- 5 In a related embodiment, the kinase comprises an amino acid sequence encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:9 or a nucleotide sequence having at least 50% similarity to all or part of the nucleotide sequence set forth in SEQ ID NO:9 or a nucleotide sequence capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:9 under low stringency conditions at 42°C.

10

The present invention further provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a novel molecule involved in or associated with regulation of cell activity and/or viability. Preferably, the nucleic acid molecule is capable of being amplified by PCR using the primers set forth in SEQ ID NO:1  
15 and/or SEQ ID NO:2.

More particularly, the present invention further provides an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:3 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:3  
20 under low stringency conditions at 42°C.

Another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:5 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:5 under low  
25 stringency conditions at 42°C.

Another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:7 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:7  
30 under low stringency conditions at 42°C

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Still another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:9 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:9 under low stringency conditions at 42°C.

5

Reference herein to a low stringency includes low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as  
10 medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and  
15 at least about 0.01M to at least about 0.15M salt for washing conditions.

Reference herein to similarity to "part" of a sequence means similarity to at least about 4 contiguous amino acids or at least about 12 contiguous nucleotide bases and more preferably at least about 7 contiguous amino acids or at least about 21 contiguous nucleotide bases.

20

The term "similarity" includes exact identity between sequences or, where the sequence differs, different amino acids may be related to each other at the structural, functional, biochemical and/or conformational levels.

25 The term "isolated" includes biological purification and biological separation and encompasses molecules having undergone at least one purification, concentration or separation step relative to its natural environment. For example, a preparation may comprise at least about 10%, preferably at least about 20%, more preferably at least about 30%, still more preferably at least about 50% or greater of the molecule relative to at least one other component in a composition  
30 as determined by activity, mass, amino acid content, nucleotide content or other convenient means.

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Hereinafter, the molecules of the present invention are referred to as a "proteinase/kinase". The term "proteinase/kinase" includes the serine proteinases HELA2 (testisin) and ATC2 and the kinase BCON3. The proteinase/kinase of the present invention may be in isolated, naturally occurring form or recombinant or synthetic form or chemical analogues thereof.

5

The proteinase/kinase of the present invention is preferably of human origin but from non-human origins are also encompassed by the present invention. Non-human animals contemplated by the present invention include primates, livestock animals (e.g. sheep, cows, pigs, goats, horses, donkeys), laboratory test animals (e.g. mice, rats, guinea pigs, hamsters, rabbits), domestic  
10 companion animals (e.g. dogs, cats), birds (e.g. chickens, geese, ducks and other poultry birds, game birds, emus, ostriches) and captive wild or tamed animals (e.g. foxes, kangaroos, dingoes). The present invention also encompasses a proteinase/kinase homologue from *Xenopus* and plants.

15 The nucleic acid molecules encoding a proteinase/kinase may be genomic DNA, cDNA or RNA such as mRNA.

Yet another aspect of the present invention provides an isolated serine proteinase encoded by a gene proximal to a cluster of genes on a mammalian chromosome. The cluster of genes is  
20 preferably on human chromosome 16p13.3 or its equivalent in a non-human species. The cluster is made up of genes all encoding or having the potential to encode a serine proteinase or homologue, derivative or functional or evolutionary equivalent thereof. Preferably, the gene cluster comprises two or more of genes comprising a nucleotide sequence selected from SEQ ID NO:3 and 5 (HELA2, short and long forms, respectively) and SEQ ID NO:28 (SP001LA),  
25 SEQ ID NO:29 (SP002LA), SEQ ID NO:30 (SP003LA) and SP004LA (see Figure 19) or a nucleotide sequence having at least 50% similarity to any one of those sequences or capable of hybridizing to any one of those sequences under low stringency conditions at 42°C.

The term "proximal" is used in its broadest sense to mean a gene cluster and includes a gene  
30 within proximity to another gene.

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Another aspect of the present invention contemplates a method for cloning a nucleotide sequence encoding a novel serine proteinase, said method comprising screening a nucleic acid library with said one or more or oligonucleotides defined by SEQ ID NO:1 and/or SEQ ID NO:2 and obtaining a clone therefrom which encodes said novel serine proteinase or part thereof.

5

Preferably, the nucleic acid library is genomic DNA, cDNA, genomic or mRNA library.

Preferably, the nucleic acid library is a cDNA expression library.

- 10 Preferably, the nucleic acid library is of human origin such as from brain, liver, kidney, neo-natal tissue, embryonic tissue, tumour or cancer tissue.

With respect to HELA2 (HELA2 (testisin)), significant expression is generally only found in normal testis. Accordingly, the present invention extends to nucleic acid molecules capable of  
15 tissue-specific or substantially tissue-specific expression.

Still another embodiment contemplates the promoter or a functional part thereof of the genomic gene encoding the subject proteinase/kinase of the present invention. The promoter may readily be obtained by, for example, "chromosome walking". A particularly useful promoter is from  
20 HELA2 (testisin) which can be regarded as a testis specific promoter. This promoter can be used, for example, to direct testis specific expression of genetic sequences operably linked to the promoter and may be used *inter alia* gene therapy or modulation of fertility.

The present invention further contemplates a range of derivatives of the subject  
25 proteinase/kinase. Derivatives include fragments, parts, portions, mutants, homologues and analogues of the subject polypeptides and corresponding genetic sequences. Derivatives also include single or multiple amino acid substitutions, deletions and/or additions to the subject molecules or single or multiple nucleotide substitutions, deletions and/or additions to the genetic sequence encoding the molecules. "Additions" to amino acid sequences or nucleotide sequences  
30 include fusions with other peptides, polypeptides or proteins or fusions to nucleotide sequences.

Reference herein to the serine proteinase and kinase includes reference to all derivatives thereof

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including functional derivatives or immunologically interactive derivatives.

Analogues of the subject serine proteinase and kinase contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with  $\text{NaBH}_4$ ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with  $\text{NaBH}_4$ .

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides.

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Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with  
5 iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine,  
10 sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 3.

TABLE 3

Non-conventional amino acid	Code	Non-conventional amino acid	Code
5 $\alpha$ -aminobutyric acid	Abu	L-N-methylalanine	Nmala
$\alpha$ -amino- $\alpha$ -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
aminocyclopropane-	Cpro	L-N-methylasparagine	Nmasn
carboxylate		L-N-methylaspartic acid	Nmasp
10 aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-	Norb	L-N-methylglutamine	Nmgln
carboxylate		L-N-methylglutamic acid	Nmglu
cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
15 D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Das	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20 D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
25 D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
30 D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva



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	D-tyrosine	Dtyr	$\alpha$ -methyl-aminoisobutyrate	Maib
	D-valine	Dval	$\alpha$ -methyl- $\gamma$ -aminobutyrate	Mgab
	D- $\alpha$ -methylalanine	Dmala	$\alpha$ -methylcyclohexylalanine	Mchexa
	D- $\alpha$ -methylarginine	Dmarg	$\alpha$ -methylcyclopentylalanine	Mcpen
5	D- $\alpha$ -methylasparagine	Dmasn	$\alpha$ -methyl- $\alpha$ -naphthylalanine	Manap
	D- $\alpha$ -methylaspartate	Dmasp	$\alpha$ -methylpenicillamine	Mpen
	D- $\alpha$ -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- $\alpha$ -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- $\alpha$ -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
10	D- $\alpha$ -methylisoleucine	Dmile	N-amino- $\alpha$ -methylbutyrate	Nmaabu
	D- $\alpha$ -methyllleucine	Dmleu	$\alpha$ -naphthylalanine	Anap
	D- $\alpha$ -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- $\alpha$ -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- $\alpha$ -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
15	D- $\alpha$ -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- $\alpha$ -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- $\alpha$ -methylserine	Dmser	N-cyclobutylglycine	Nebut
	D- $\alpha$ -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D- $\alpha$ -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
20	D- $\alpha$ -methyltyrosine	Dmtty	N-cyclodecylglycine	Ncdec
	D- $\alpha$ -methylvaline	Dmval	N-cyclododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
25	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
30	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
	D-N-methyllleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp

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	D-N-methyllysine	Dnmlys	N-methyl- $\gamma$ -aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
5	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyl- $\alpha$ -naphthylalanine	Nmanap
10	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	$\gamma$ -aminobutyric acid	Gabu	N-( <i>p</i> -hydroxyphenyl)glycine	Nhtyr
	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L- $\alpha$ -methylalanine	Mala
15	L- $\alpha$ -methylarginine	Marg	L- $\alpha$ -methyloasparagine	Masn
	L- $\alpha$ -methyloaspartate	Masp	L- $\alpha$ -methyl- <i>t</i> -butylglycine	Mtbug
	L- $\alpha$ -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- $\alpha$ -methylglutamine	Mgln	L- $\alpha$ -methylglutamate	Mglu
	L- $\alpha$ -methylhistidine	Mhis	L- $\alpha$ -methylhomophenylalanine	Mhphe
20	L- $\alpha$ -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L- $\alpha$ -methylleucine	Mleu	L- $\alpha$ -methyllysine	Mlys
	L- $\alpha$ -methylmethionine	Mmet	L- $\alpha$ -methylnorleucine	Mnle
	L- $\alpha$ -methylnorvaline	Mnva	L- $\alpha$ -methylornithine	Morn
	L- $\alpha$ -methylphenylalanine	Mphe	L- $\alpha$ -methylproline	Mpro
25	L- $\alpha$ -methylserine	Mser	L- $\alpha$ -methylthreonine	Mthr
	L- $\alpha$ -methyltryptophan	Mtrp	L- $\alpha$ -methyltyrosine	Mtyr

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L- $\alpha$ -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
carbamylmethyl)glycine		carbamylmethyl)glycine	
1-carboxy-1-(2,2-diphenyl-	Nmbc		
5 ethylamino)cyclopropane			

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having  $(CH_2)_n$  spacer groups with  $n=1$  to  $n=6$ ,  
 10 glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of  $C_\alpha$  and  $N_\alpha$ -methylamino acids, introduction of double bonds between  $C_\alpha$  and  $C_\beta$  atoms of amino acids and  
 15 the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

These types of modifications may be important to stabilise the proteinase/kinase if administered  
 20 to an individual or for use as a diagnostic reagent.

The present invention further contemplates chemical analogues of the proteinase/kinase capable of acting as antagonists or agonists of the native molecules or which can act as functional analogues of the native molecules. For example, an antagonist may be a proteinase inhibitor.  
 25 Chemical analogues may not necessarily be derived from the subject enzymes but may share certain conformational similarities. Alternatively, chemical analogues may be specifically designed to mimic certain physiochemical properties of the serine proteinases or kinases. Chemical analogues may be chemically synthesised or may be detected following, for example, natural product screening.

30

The identification of the novel molecules of the present invention permits the generation of a

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range of therapeutic molecules capable of modulating expression of their native counterparts or modulating their activity. Modulators contemplated by the present invention includes agonists and antagonists of proteinase/kinase expression. Antagonists of proteinase/kinase expression include antisense molecules, ribozymes and co-suppression molecules. Agonists  
5 include molecules which increase promoter ability or interfere with negative regulatory mechanisms. Agonists of proteinase/kinase include molecules which overcome any negative regulatory mechanism. Antagonists of the proteinase/kinase include antibodies and inhibitor peptide fragments.

10 Other derivatives contemplated by the present invention include a range of glycosylation variants from a completely unglycosylated molecule to a modified glycosylated molecule. Altered glycosylation patterns may result from expression of recombinant molecules in different host cells.

15 Another embodiment of the present invention contemplates a method for modulating expression of proteinase/kinase in a human, said method comprising contacting the proteinase/kinase gene encoding proteinase/kinase with an effective amount of a modulator of proteinase/kinase expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of proteinase/kinase. For example, a nucleic acid  
20 molecule encoding proteinase/kinase or a derivative thereof may be introduced into a cell conversely, proteinase/kinase antisense sequences such as oligonucleotides may be introduced.

Another aspect of the present invention contemplates a method of modulating activity of proteinase/kinase in a human, said method comprising administering to said mammal a  
25 modulating effective amount of a molecule for a time and under conditions sufficient to increase or decrease proteinase/kinase activity. The molecule may be a proteinaceous molecule or a chemical entity and may also be a derivative of proteinase/kinase or its receptor or a chemical analogue or truncation mutant of proteinase/kinase or its receptor.

30 One particularly useful serine proteinase, HELA2 (testisin), is implicated in spermatogenesis and in testicular tumour development. It is proposed, in accordance with the present invention,

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that HELA2 (testisin) is involved in fertility and infertility.

Northern blot analysis of Poly A+ RNA from normal tissue specimens showed a unique tissue distribution for HELA2 (testisin) with significant expression only in the testis. No signals are  
5 detected in any other tissue, with the exception of a minor signal in salivary gland. By RT-PCR, HELA2 (testisin) is detected in the ejaculate of normal males but not in the ejaculate of vasectomised males indicating that it is of germ cell origin. Hybridization data *in situ* indicated that HELA2 (testisin) is produced by immature germ cells in the testis, located near the basal epithelium and, hence, is an important factor for normal sperm maturation; defective expression  
10 or mutations would contribute to primary male infertility. Further, it is from the precursors of spermatocytes that 95% of testicular germ cell tumours, such as seminomas, embryonal carcinomas and teratocarcinomas arise. In the normal testis, germ cells undergo meiosis to become spermatocytes, but in individuals at risk, the germ cells continue to proliferate giving rise to germ cell tumours. Although not wishing to limit the present invention to any one theory  
15 or mode of action, it is proposed, in accordance with present invention, that HELA2 (testisin) functions at this critical juncture - cell growth versus maturation.

Familial forms of testicular cancer are rare, but linkage analysis of a large family with familial seminoma has demonstrated linkage to chromosome 16p, within a region adjacent to the  
20 HPKD1 (human polycystic kidney disease) gene at 16p13.3. The HELA2 (testisin) gene localises to chromosome 16p13.3 which is near the telomere of chromosome 16 and is associated with high genetic instability. The HELA2 (testisin) gene is sandwiched between four genes which underlie other human genetic disorders; HPKD1 and tuberous sclerosis (TSC2) on the one side, and familial mediterranean fever (MEF) and Rubenstein-Taybi syndrome  
25 (RSTS) on the other side. The question of whether HELA2 (testisin) may be a tumour suppressor for seminoma was determined by comparing HELA2 (testisin) mRNA expression in normal testes with corresponding germ cell tumours from patients with seminoma. HELA2 (testisin) was not detectable in the tumours of these patients, but was present in the corresponding normal testis specimens, indicative of a tumour suppressor role of HELA2  
30 (testisin) in testicular germ cell cancers.

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Although restricted in normal tissues to the testes, HELA2 (testisin) is expressed in tumours of the colon, pancreas, prostate and ovary. This indicates that HELA2 (testisin) contributed to tumourigenesis and, therefore, has an application as a marker and also as a therapeutic anti-tumour target in these types of cancers.

5

These data point to a potentially very significant role for HELA2 (testisin) in testicular germ cell maturation (spermatogenesis) as well as in the genesis of testicular germ cell tumours. In accordance with the present invention, it is proposed that expression of HELA2 (testisin) by immature germ cells may be essential for sperm cell development, such that loss of HELA2  
10 (testisin) expression leads to continued and uncontrolled proliferation of immature germ cells leading to subsequent tumourigenesis. Germ cells wherein HELA2 (testisin) is mutated or absent may thus be prone to malignant transformation because of an inability to progress along the differentiation pathway.

15 HELA2 (testisin) is well-positioned to anchor on the surface of the germ cell where it would participate in a range of proteolytic activities, including cell migration, differentiation and/or activation of growth factors, receptors, or cytokines as well as initiate additional proteolytic cascades. Although not intending to limit the present invention to any one theory or mode of action, it is proposed, in accordance with the present invention, that the proteolytic target of  
20 HELA2 (testisin) is a cytokine, receptor or growth factor essential for either germ cell proliferation or differentiation - ie. HELA2 (testisin) may either inactivate a factor important for proliferation, or activate a factor which promotes differentiation. Thus, HELA2 (testisin) may be critical in the regulation of specific cytokines, cytokine receptors or growth factors by means of post-translational proteolytic processing. That HELA2 (testisin) is not present in  
25 other normal tissues of the male urogenital tract, such as the prostate and kidney, also argues for such a role specific to the testis.

Diagnostic and therapeutic applications for HELA2 (testisin) have the potential to be wide-ranging both in the cancer and fertility/infertility markets. In tumours, other than the testis, it  
30 is desirable to block or inhibit HELA2 (testisin) activity. As HELA2 (testisin) is a member of the serine proteinase family, for which prototype crystal structures are known and the catalytic

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mechanism reasonably well characterised, the design of drugs that target HELA2 (testisin) proteolytic activity as an anti-tumour therapy should be relatively straightforward. As HELA2 (testisin) is predicted to be anchored on the cell surface, there would not be difficulties associated with delivery of drugs to intracellular compartments. Further, it is very possible that  
5 some tumour-associated HELA2 (testisin) may be proteolytically cleaved from the surface of tumour cells, and the extracellular domain detectable in patient serum as a potential tumour associated marker.

Testicular cancer is the commonest malignancy in men aged 20-44 years. Early diagnosis  
10 correlates with an improved chance of cure and in a reduction in the severity of treatment. If the cancer is not treated early, it becomes very aggressive. The incidence of testicular cancer is significant (9/100,000) and has been rising over the last 10 years. In testicular germ cell tumours, such as seminoma, delivery of recombinant HELA2 (testisin) using gene therapy techniques could lead to arrest of tumour growth and potentially allow commencement of  
15 normal sperm cell maturation and differentiation, thereby reducing the need for surgical removal of the testis (orchidectomy). This may be particularly effective for patients who have already had one testicle removed because of testicular cancer. The risk of contralateral testicular cancer is increased in these patients and tumour development could be arrested through early treatment with HELA2 (testisin) to arrest growth and assist maturation of germ cells. The finding of  
20 mutant forms of HELA2 (testisin) may also lead to new markers for seminoma. Unlike other testicular non-seminoma cancers where  $\alpha$ -fetoprotein and  $\beta$ -HCG are frequently elevated and can be used as tumour markers, the lack of an adequate marker for seminoma creates difficulties with staging and patient follow-up.

25 A demonstrated role for HELA2 (testisin) in sperm maturation and development would likely lead to improved diagnosis and new directed therapeutics for male primary infertility. Primary male infertility is responsible for conception problems in 5-10% of couples and the world market for a therapeutic in this area would be very substantial. Delivery of recombinant HELA2 (testisin) could assist sperm maturation and potentially trigger normal sperm  
30 development in some of these cases. The identification of mutant forms of HELA2 (testisin) could aid in diagnosis of infertility. If HELA2 (testisin) does not prove to be a tumour

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suppressor, but is important for sperm maturation, it could provide a new target for the development of a male contraceptive. If hormonal regulation of HELA2 (testisin) can be demonstrated, HELA2 (testisin) may prove effective for the treatment of conditions arising from dysfunctional hormonal responses, such as cryptorchidism, which is associated with both  
5 infertility and seminoma development.

Accordingly, the present invention contemplates a pharmaceutical composition comprising proteinase/kinase or a derivative thereof or a modulator of proteinase/kinase expression or proteinase/kinase activity and one or more pharmaceutically acceptable carriers and/or diluents.  
10 These components are referred to as the "active ingredients" and include, for example, HELA2 (testisin).

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable  
15 solutions. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the  
20 use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimersal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of  
25 the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as  
30 required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion



medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

5

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be  
10 incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful  
15 compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1  $\mu$ g and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed  
20 hereafter. A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of  
25 the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any  
30 dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release

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preparations and formulations.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and  
5 the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

- 10 It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Parental compositions are generally suitable for administration by the intravenous, subcutaneous or intramuscular routes amongst other routes of administration. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined  
15 quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living  
20 subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail. Other forms of administration include but are not limited to intranasal, buccal, rectal, suppository, inhalation, intracerebral and intraperitoneal.

The principal active ingredient is compounded for convenient and effective administration in  
25 effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5  $\mu$ g to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5  $\mu$ g to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are  
30 determined by reference to the usual dose and manner of administration of the said ingredients.

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The effective amounts include amounts calculated or predicted to have the desired effect and range from at least about 0.01 ng/kg body weight to about 10,000 mg/kg body weight. Alternative amounts include 0.1 ng/kg body weight to about 1000 ng/kg body weight.

- 5 The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating proteinase/kinase expression or proteinase/kinase activity. The vector may, for example, be a viral vector. This form of therapy is proposed to be particularly useful for gene replacement or enhancement therapy for HELA2 (testisin) especially for the modulation of
- 10 fertility and/or treatment of testicular cancer.

Still another aspect of the present invention is directed to antibodies to proteinase/kinase and its derivatives. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to proteinase/kinase or may be specifically raised to

15 proteinase/kinase or derivatives thereof. In the case of the latter, proteinase/kinase or its derivatives may first need to be associated with a carrier molecule. The antibodies and/or recombinant proteinase/kinase or its derivatives of the present invention are particularly useful as therapeutic or diagnostic agents. For example, monitoring non-testicular cancer by measuring HELA2 (testisin) or screening for the presence of testicular cancer by an absence of

20 HELA2 (testisin).

Proteinase/kinase and its derivatives may also be used to screen for naturally occurring antibodies to proteinase/kinase. These may occur, for example in some autoimmune diseases. Alternatively, specific antibodies can be used to screen for proteinase/kinase. Techniques for

25 such assays are well known in the art and include, for example, sandwich assays and ELISA. Knowledge of proteinase/kinase levels may be important for diagnosis of certain cancers or a predisposition to cancers or for monitoring certain therapeutic protocols.

Antibodies the proteinase/kinase of the present invention may be monoclonal or polyclonal.

30 Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A

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"synthetic antibody" is considered herein to include fragments and hybrids of antibodies. The antibodies of this aspect of the present invention are particularly useful for immunotherapy and may also be used as a diagnostic tool for assessing apoptosis or monitoring the program of a therapeutic regimen.

5

For example, specific antibodies can be used to screen for proteinase/kinase proteins. The latter would be important, for example, as a means for screening for levels of proteinase/kinase in a cell extract or other biological fluid or purifying proteinase/kinase made by recombinant means from culture supernatant fluid. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies or synthetic antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of proteinase/kinase.

Both polyclonal and monoclonal antibodies are obtainable by immunization with the enzyme or protein and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of proteinase/kinase, or antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoabsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques

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which are well known to those who are skilled in the art.

Another aspect of the present invention contemplates a method for detecting proteinase/kinase in a biological sample from a subject said method comprising contacting said biological sample  
5 with an antibody specific for proteinase/kinase or its derivatives or homologues for a time and under conditions sufficient for an antibody-proteinase/kinase complex to form, and then detecting said complex.

The presence of proteinase/kinase may be accomplished in a number of ways such as by  
10 Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These, of course, includes both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

15

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought  
20 into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the  
25 antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled  
30 in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain proteinase/kinase including cell extract,

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tissue biopsy or possibly serum, saliva, mucosal secretions, lymph, tissue fluid and respiratory fluid. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and supernatant fluid such as from a cell culture.

5 In the typical forward sandwich assay, a first antibody having specificity for the proteinase/kinase or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface  
10 suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g. from  
15 about room temperature to about 37°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

20

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody.  
25 Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its  
30 chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most

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commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionucleotide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a

5 wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding

10 phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme

15 linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

20 Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent

25 labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as

30 radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

The present invention also contemplates genetic assays such as involving PCR analysis to detect proteinase/kinase gene or its derivatives. Alternative methods or methods used in conjunction include direct nucleotide sequencing or mutation scanning such as single stranded conformation polymorphisms analysis (SSCP), specific oligonucleotide hybridisation, and methods such as  
5 direct protein truncation tests.

The nucleic acid molecules of the present invention may be DNA or RNA. When the nucleic acid molecule is in DNA form, it may be genomic DNA or cDNA. RNA forms of the nucleic acid molecules of the present invention are generally mRNA.

10

Although the nucleic acid molecules of the present invention are generally in isolated form, they may be integrated into or ligated to or otherwise fused or associated with other genetic molecules such as vector molecules and in particular expression vector molecules. Vectors and expression vectors are generally capable of replication and, if applicable, expression in one or  
15 both of a prokaryotic cell or a eukaryotic cell. Preferably, prokaryotic cells include *E. coli*, *Bacillus sp* and *Pseudomonas sp*. Preferred eukaryotic cells include yeast, fungal, mammalian and insect cells.

Accordingly, another aspect of the present invention contemplates a genetic construct  
20 comprising a vector portion and a mammalian and more particularly a human proteinase/kinase gene portion, which proteinase/kinase gene portion is capable of encoding an proteinase/kinase polypeptide or a functional or immunologically interactive derivative thereof.

Preferably, the proteinase/kinase gene portion of the genetic construct is operably linked to a  
25 promoter on the vector such that said promoter is capable of directing expression of said proteinase/kinase gene portion in an appropriate cell.

In addition, the proteinase/kinase gene portion of the genetic construct may comprise all or part of the gene fused to another genetic sequence such as a nucleotide sequence encoding  
30 glutathione-S-transferase or part thereof.



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The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells comprising same.

The present invention also extends to any or all derivatives of proteinase/kinase including  
5 mutants, part, fragments, portions, homologues and analogues or their encoding genetic sequence including single or multiple nucleotide or amino acid substitutions, additions and/or deletions to the naturally occurring nucleotide or amino acid sequence. The present invention further encompasses hybrids between the proteinase/kinases such as to broaden the spectrum of activity and to ligands and substrates of the proteinase/kinase.

10

The proteinase/kinase and its genetic sequence of the present invention will be useful in the generation of a range of therapeutic and diagnostic reagents.

Soluble proteinase/kinase polypeptides or other derivatives, agonists or antagonists are also  
15 contemplated to be useful in the treatment of disease, injury or abnormality in the nervous system, e.g. in relation to central or peripheral nervous system to treat Cerebral Palsy, trauma induced paralysis, vascular ischaemia associated with stroke, neuronal tumours, motoneurone disease, Parkinson's disease, Huntington's disease, Alzheimer's disease, Multiple Sclerosis, peripheral neuropathies associated with diabetes, heavy metal or alcohol toxicity, renal failure  
20 and infectious diseases such as herpes, rubella, measles, chicken pox, HIV or HTLV-1. Other conditions for which the proteinase/kinase are useful include cancer, metastasis and autoimmune disease amongst many others. Particular applications for HELA2 (testisin) include as a marker for non-testicular cancers, in the treatment of testicular cancer and in the treatment of infertility or in inducing infertility such for contraception.

25

A further aspect of the present invention contemplates the use of proteinase/kinase or its functional derivatives in the manufacture of a medicament for the treatment of proteinase/kinase mediated conditions defective or deficient.

30 The present invention is further described by the following non-limiting Examples.

- 40 -

### EXAMPLE 1

#### CLONING PROCEDURES

In order to identify serine proteinases that may be involved in regulatory cellular functions, a genetic screening approach was applied using degenerate primers corresponding to conserved regions of serine proteinases (amino acids flanking His- and Ser- residues) to amplify gene fragments spanning these regions from cDNA, using a low stringency RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) approach.

By this technique, the aim was to isolate low abundance genes as well as those present in moderate to high abundance. The cDNA used for these experiments was isolated from a HeLa cell cytotoxicity model wherein PAI-2 expression inhibits TNF(-induced apoptosis (Dickinson *et al J. Biol. Chem.* 270: 27894-27904, 1995). These PAI-2 expressing cells provide a unique and viable system for investigating TNF(signalling pathways as they are protected from the cytotoxic effects of TNF).

cDNA was generated from RNA isolated from HeLa cells and PAI-2 expressing HeLa cells, both untreated and following treatment with TNF and cycloheximide. Amplification of each cDNA population using PCR and the following serine proteinase degenerate primers,

20

His Primer: 5'ACAGAATTCTGGGTIGTIACIGCIGCICAYTG3' [SEQ ID NO:1],

Ser Primer: 5'ACAGAATTCAXIGGICCCIC/GT/AXTCICCC3' [SEQ ID NO:2]

(where X= A or G; Y= C or T; I= Inosine)

produced DNA fragments in the range of 480bp, the approximate predicted size of the serine proteinase intergenic region. These amplified DNA fragments were cloned into E. coli generating a library containing approximately 150 independent clones. The inventors analysed 36 of these clones and found that 9 encoded previously identified serine proteinases or tissue-type or urokinase-type plasminogen activators, thereby demonstrating the efficacy of this approach. Of the other 36, two were found to encode novel open reading frames with high homology to serine proteinases and are referred to herein as "HELA2" (or "testisin") and

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"ATC2". One additional clone designated herein, "BCON3", showed homology to a kinase. Extension of the DNA fragments by RACE in both 5' and 3' directions using internally derived primers has verified the homology of HELA2 and ATC2 to the serine proteinase family. Each of the three DNA sequences are unique in that they are markedly different from any known DNA or protein sequence in the Genbank and NBRF databases.

## EXAMPLE 2

### HELA2 SERINE PROTEINASE (TESTISIN)

10 The HELA2 mRNA transcript is approximately 1.5kb as determined from Northern blot analysis. Nucleic acid sequence was obtained for about 1.1kb of HELA2 which spans the entire coding region, the 3' noncoding region and part of the 5' noncoding region. The coding region starts with an ATG codon which is present in a motif analogous to the Kozak eukaryotic translation initiation consensus sequence. Alignment of the deduced amino acid sequence of  
15 HELA2 with homologous serine proteinases shows that the cDNA encodes a 314 amino acid (aa) polypeptide with a calculated molecular weight of 34.8kD (called Testisin), which is synthesized as a zymogen containing pre-, pro- and catalytic regions (Figure 1). The pro- region (or light chain) and the catalytic region (heavy chain) are delineated by a classic serine proteinase activation motif Arg-Ile-Val-Gly-Gly [SEQ ID NO:24] with cleavage likely  
20 occurring between Arg and Ile. The catalytic region includes the catalytic triad of His, Asp and Ser in positions and motifs which are highly conserved among the serine proteinases. Ten Cys residues occur in conserved positions: by analogy to other serine proteinases, eight of these function to form disulfide bridges within the catalytic region and the remaining two link the pro- and catalytic regions.

25

Structural features conserved in the binding pockets of serine proteinases are present in HELA2 (testisin). An Asp residue at the bottom of the serine proteinase binding pocket six residues before the active site Ser in HELA2 (testisin) indicates that HELA2 (testisin) has trypsin-like specificity, with proteolytic cleavage after Arg or Lys in target substrates. HELA2 (testisin) also  
30 contains a conserved Ser-Trp-Gly motif at the top of the binding pocket which is likely involved in hydrogen bonding with target substrates in other serine proteinases.

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A hydrophobicity plot of the HELA2 (testisin) amino acid sequence (Figure 1) identifies two hydrophobic regions, one located at the amino terminus and the other at the carboxy terminus. The 20 aa amino terminal hydrophobic region is likely to be a signal peptide, which would direct newly synthesized HELA2 to enter the endoplasmic reticulum. The 16 aa hydrophobic carboxy terminus of HELA2 (testisin) shows high homology to the transmembrane domain of prostasin (Figure 2), suggesting that HELA2 (testisin) is likely to be a membrane-anchored serine proteinase. Thus HELA2 (testisin) may anchor on the germ cell surface where it could participate in a range of proteolytic activities, including participation in cell migration, differentiation and/or activation of growth factors and proteolytic cascades. In prostasin, this protruding carboxy terminus may be cleaved, thus releasing the serine proteinase from the membrane. A similar cleavage event may also occur with Testasin.

Two isoforms of HELA2 were identified in a HeLa cell cDNA library (Stratagene UniZap HeLa Library) which differ by an insertion of 6 nucleotides which generates a SfiI restriction enzyme site. At the protein level, there is a corresponding insertion of 2 aa's (Tyr-Ser) within the catalytic binding pocket (Figure 2A). The two isoforms of HELA2 cDNA are referred to as the short (S) and long (L) isoforms, respectively. The nucleotide and corresponding amino acid sequence for the short isoform of HELA2 is shown in SEQ ID NOs. 3 and 4, respectively. The long isoform is shown in SEQ ID NO:5 and 6, respectively.

20

### EXAMPLE 3

#### GENERATION OF FULL LENGTH cDNA ENCODING HELA2 (TESTISIN)

Partial cDNA fragments of the short and long isoforms of HELA2 were obtained using a combination of library screening techniques. Plasmids containing the full length cDNA of the two isoforms were then generated in pBluescriptSK(-) by ligating restriction enzyme-digested fragments of the partial cDNAs. A plasmid map of the two generated constructs, pBluescriptHELA2(S) and pBluescriptHELA2(L), and a restriction enzyme map of the long isoform cDNA are diagrammed in Figure 3.

30

In vitro transcription/translation using HELA2 cDNA shows a major specific product of

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approximately 35kD (Fig. 2B), which is the same as size predicted from the open reading frame, demonstrating that HELA2 cDNA encodes a protein. The translation/transcription coupled rabbit reticulocyte lysate system (Promega) was used as per the manufacturer's instructions for 35S-methionine labelling. Clones of HELA2 in pBluescript a PAI-2 positive control were used  
5 with T3-RNA polymerase (sense direction).

#### EXAMPLE 4

##### EXPRESSION OF RECOMBINANT HELA2 (TESTISIN) IN E.COLI

###### 10 (A) Generation of expression constructs

###### (i) His(6)-tagged recombinant HELA2 (testisin)

To reduce potential toxic effects on host cells, and therefore optimise expression, a strategy was employed to eliminate the hydrophobic residues of the secretory and membrane anchoring domains of HELA2 (testisin) (Testisin (20-295)). Testisin (20-295) fragments which were His6  
15 tagged at either the amino or carboxy terminal were obtained by PCR and expression constructs were generated by inserting these into pQE vectors (Qiagen).

The primers used to generate the amino-terminal tagged protein were:

forward: 5' GCACAGTCGACCAAGCCGGAGTCGCAGAG 3' [SEQ ID NO:11] and

20 reverse: 5' GCACAAAGCTTGCCAGGAGGGGTCTGGCTG 3' [SEQ ID NO:12]

The amplification product of 858bp was digested with SalI and HindIII and ligated into pQE-10 to give pQE-10(20-295)N (Figure 4).

The primers used to generate the carboxy-terminal tagged protein were:

25 forward: 5' GCACAACCATGGCCAAGCCGGAGTCGCAGGAG 3' [SEQ ID NO:13] and

reverse 5' GCACAAGATCTCCAGGAGGGGTCTGGCTG 3' [SEQ ID NO:14].

The amplification product of 859 bp was digested with NcoI and BgIII and ligated into PQE-60 to give pQE-60(20-295)C (Figure 4).

###### 30 (ii) GST-tagged recombinant HELA2 (testisin)

In order to generate a fusion of glutathione-S-transferase (GST) and HELA2 (testisin),

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pBluescriptHELA2(S) was digested with Sau3A1, releasing a 570bp DNA fragment encoding the 190 amino acids at the carboxy terminal end of HELA2 (testisin). This DNA fragment was cloned into the BamH1 site of pGEX-1 generating pGEX-1(90-279) (Figure 4) and subjected to DNA sequence analysis to confirm that the fusion was in frame.

5

(b) Expression of His-tagged HELA2 (testisin) in *E. coli*

pQE10(20-295)N and pQE60(20-295)C plasmids were electro-transformed into *E. coli* DH5( cells. Four different clones were selected for further analysis: His-N21 expressing amino  
10 terminal His6-tagged Testisin (20-295); and His-C21, His-C22, and His-C23 expressing carboxy terminal His6-tagged Testisin (20-295). To express recombinant HELA2 (testisin) protein, transformed cells were grown to log phase then induced for 4 hours in the presence of 2mM IPTG. Cells were lysed in a denaturing lysis buffer containing 8M urea, 0.1M NaH<sub>2</sub>PO<sub>4</sub> and 0.01M Tris/HCl pH8. Alternatively the cells were lysed in a non-denaturing lysis buffer  
15 containing 0.1M NaH<sub>2</sub>PO<sub>4</sub>, 0.1M NaCl and 0.01 M Tris/HCl pH8. The His6 tagged protein was recovered by mixing the lysate with a metal affinity resin (Qiagen or Clontech). Purified testisin(L) was eluted with 100 mM EDTA in lysis buffer (pH 6.3). A major band of approximately 32 kDa was obtained in the eluate as shown by the arrows in Figure 5A. Western blot analysis of a purification of the His-C23 clone using an anti-His6 antibody showed  
20 that the band at 32 kDa was His6 tagged HELA2 (testisin) (Figure 5B).

## EXAMPLE 5

### IMMUNOLOGY

25 (A) Rabbit Polyclonal Antibodies Directed Against HELA2 (testisin) Peptide Antigens

Three peptides were selected from the HELA2 (testisin) amino acid sequence on the basis of predicted antigenicity, hydrophilicity and lack of identity with known proteins (Figure 6).

30 Peptide antigen T20-33

KPESQEAAPLSGPC [SEQ ID NO:15]

Peptide antigen T46-63

EDAELGRWPWQGSLRLWDC [SEQ ID NO:16]

- 45 -

Peptide antigen T175-190 GYIKEDEALPSPHTLQC [SEQ ID NO:17]

These peptides were synthesized (Auspep) and coupled to keyhole limpet hemocyanin. The coupled peptide (500 Fg) in PBS (0.5 ml) was emulsified in an equal volume of Freund's complete adjuvant before injection into a rabbit. Booster injections of coupled peptide in Freund's incomplete adjuvant were made at intervals of 2 to 3 weeks. Each rabbit was bled (approximately 1 ml) before the initial injection and about 7 days after the second and subsequent boosters and the antibody titre assessed by direct ELISA assay. Immunoreactive antisera against the peptide antigens was demonstrated and when a sufficiently high titre was achieved (after 3 to 5 boosters), between 12 and 25 ml of blood was removed from each animal.

Rabbit antisera was affinity purified against the respective immunising peptides by chromatography using peptide-coupled affinity columns. Immunoreactivity of the affinity purified antibodies against HELA2 (testisin) was demonstrated by Western blot analysis of GST-tagged recombinant HELA2 (testisin). pGEX-1(90-279) plasmid DNA (described in Example 4) was electro-transformed into E. coli DH5( cells and induced for 3 hours in the presence of 0.5mM IPTG. Cells were lysed in 1.5% sarcosyl, 2% Triton X100 and then sonicated. After removal of the insoluble fraction by centrifugation, the cell lysate was mixed with a 50% slurry of Glutathione Sepharose 4B, washed, and the purified GST-Testisin(90-279) was eluted by boiling with SDS-Sample buffer. Figure 7 shows an example of Western blot analysis of the eluate using anti-Peptide T175-190 antibody demonstrating a purified, immunoreactive band representative of GST-linked HELA2(testisin) of approximately 47 kDa.

(B) Rabbit Polyclonal Antibodies Directed Against Purified Bacterially Expressed HELA2 (testisin)

An SDS-PAGE gel slice containing purified His6 tagged HELA2 (testisin) (as described in Example 4, part (b)) is to be combined with adjuvant and rabbits immunized as described above. Rabbit antisera are tested by Western blot analysis for immunoreactivity against purified recombinant HELA2 (testisin) and HELA2 (testisin) in cell extracts, as well as use in immunohistochemical analyses.

## EXAMPLE 6

### EXPRESSION OF HELA2 (TESTISIN) IN EUKARYOTIC CELLS

#### (A) Generation of expression constructs

5

Eukaryotic expression constructs encoding testisin(s) and testisin(L) His6 tagged at the carboxy terminal were generated in the eukaryotic expression vector pcDNA3 (Invitrogen). DNA fragments encoding HELA2 (testisin) were generated by PCR from both pBluescriptHELA2(S) and pBluescriptHELA2(L) using the primers:

10 forward: 5' GCACAGGTACCGAGGCCATGGGCGCGCGC 3' [SEQ ID NO:18] and  
reverse 5' GCACATCTAGATCAGTGGTGGTGGTGGTGGTGGACCGGCCCCAGGA  
GTGG 3' [SEQ ID NO:19]

The PCR product of 985 bp obtained from amplification of HELA2 (testisin) from  
15 pBluescriptHELA2(S) as template was ligated into pGEM-T (Easy) vector (Promega).  
Digestion of this shuttle construct with NotI released a 1025 bp fragment which was ligated into  
pcDNA3 generating the short isoform expression construct pcDNA3-Test(S-C) (Figure 8).  
PCR amplification of the long isoform template gave a 991 bp product which was ligated into  
pGEM-T (easy) vector. NotI digestion of the shuttle construct released a 1031 bp fragment  
20 which was ligated into pcDNA3 giving pcDNA3-Test(L-C) (Figure 8).

Soluble testisin (1-295)-His6 in which the membrane anchoring sequence is deleted and the  
protein is carboxy-His6 tagged is to be obtained by PCR amplification of HELA2 (testisin) from  
pBluescriptHELA2(L) using the primers:

25 forward: 5' GCACAGCGGCCGCGAGGCCATGGGCGCGCGC 3' [SEQ ID NO:20] and  
reverse: 5' GCACAGCGGCCGCTCAGTGGTGGTGGTGGTGGTGGCCAGGAGGGGTC  
TGGCTG 3' [SEQ ID NO:21].

The PCR product will be digested with NotI and ligated into pcDNA3 generating the long  
isoform expression construct pcDNA3-Test(1-295)L-C (Figure 8).

30

#### (B) Expression and cellular localisation of HELA2 (testisin)



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Each of the expression constructs is transiently transfected into a eukaryotic cell line (eg. HeLa, CHO or COS cells) by electroporation. Expression is confirmed by Northern blot and immunoblot. The His6 tag is a small, uncharged tag which reportedly does not interfere with cellular membrane interactions and is able to be detected with anti-His6 antibodies. HELA2 (testisin) cellular localisation is analysed by immunofluorescence using antibodies directed against the His6 tag and stained cells examined by confocal microscopy. Mock transfected cells is monitored as one of the controls in these experiments. Cells are examined under non-permeabilised and permeabilised conditions to investigate intracellular and cell surface expression of HELA2 (testisin) tagged proteins. Possible release of HELA2 (testisin) into the supernatant is monitored by immunoblotting of conditioned media. Association of HELA2 (testisin) with a particular cellular compartment is confirmed by cellular fractionation studies. Stable transfectants of the full length and truncated tagged HELA2 (testisin) is generated by selection in G418. Recombinant HELA2 (testisin) is purified from these stable transfectants using a metal affinity resin (eg. Qiagen or Clontech) for assay of its bioactivity and efficacy as a therapeutic reagent.

### EXAMPLE 7

#### HELA2 (TESTISIN) IS SPECIFICALLY EXPRESSED IN THE NORMAL TESTIS, AND IS ASSOCIATED WITH SPERM DEVELOPMENT

20

##### (A) Normal Tissue Blot

Dot blot analysis of PolyA+ RNA from 50 normal tissue specimens (standardised to 8 different housekeeping genes) (Clontech) was performed using a 32P-labelled HELA2 (testisin) probe. Hybridization of the radiolabelled probe was in ExpressHyb solution (Clontech) at 65°C. The blots were washed to a final stringency of 0.1xSSC/0.5% w/v SDS. High level expression of HELA2 (testisin) was found only in the testis as shown by the histogram plot of the Signal Intensity in Figure 9. In contrast, probing of the same blot with BCON3 showed ubiquitous expression of BCON3 mRNA in a variety of tissues (Figure 9).

30

##### (B) Multiple Tissue Northern Blot

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Northern blots displaying polyA+ mRNA from 16 different normal tissues (Clontech) were hybridised at 65°C in ExpressHyb solution using a 400bp SacII/EcoRI 32P-labelled HELA2 probe for 3h and then washed to a final stringency of 0.1xSSC/0.1%SDS at 60°C. After a 5h exposure, a strong band was observed only in the lane containing testis mRNA, demonstrating the specificity of HELA2 (testisin) expression for the testis (Figure 10A). Prolonged exposure (4.5 days) of the blot revealed a very low level of HELA2 (testisin) mRNA expression in the prostate, lung and pancreas only. In contrast to HELA2, BCON3 is expressed in mRNA from most tissues present on the blot (Figure 10B).

10

(C) HELA2 (testisin) is Expressed in Sperm Cells, Demonstrating its Germ Cell Origin

To determine whether HELA2 (testisin) expression is associated with germ cells of the testis, ejaculate specimens from normal fertile males were compared with those of post-vasectomy males by RT-PCR analysis using HELA2 (testisin) specific primers. Sperm is the primary product from the testis that is found in ejaculate; other components of the ejaculate are derived from the prostate.

First strand cDNA was reverse transcribed from total RNA which has been isolated from frozen or fresh ejaculate specimens. PCR was performed on the cDNA templates using the primers: forward: 5' CTGACTTCCATGCCATCCTT 3' [SEQ ID NO:22] and reverse: 5' GCTCACGACTCCAATCTGAT 3' [SEQ ID NO:23].

As shown in Figure 11, strong signals of the expected size of 464 bp were detected in ejaculate from normal males (Patients #23 and #31), while no HELA2 (testisin) was detected in Patient #153 (post-vasectomy). Patient #90 (post-vasectomy) showed a low level of amplification product which may reflect a small amount of residual sperm in the seminiferous tubules. PCR using primers specific for (2-macroglobulin was performed on the same samples as a control for the presence of approximately equal amounts of cDNA in each sample.

30 (D) HELA2 (testisin) is Expressed in Immature Germ Cells of the Testis

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In situ hybridization was performed on paraffin-embedded specimens of rat testis tissue using DIG- labelled HELA2 (testisin) RNA probes (T3 and T7 generated transcripts containing nucleotides 1-423 of HELA2 cDNA). The results using the antisense RNA probe showed strong positive staining near the basal lamina of the seminiferous tubules in the region associated with spermatocytes and spermatogonia (Figure 12, see arrows). HELA2 (testisin) mRNA expression did not appear to be associated with Leydig cells and the pattern was not typical for Sertoli cell staining. The presence of HELA2 (testisin) mRNA in these cells indicates a role for HELA2 (testisin) in germ cell maturation and sperm development.

10

#### EXAMPLE 8

#### HELA2 (TESTISIN) EXPRESSION IS ASSOCIATED WITH TUMOURS IN NON-TESTIS CELL-TYPES

15 The tissue and cell-type distribution of testisin mRNA transcripts in tumours were determined by Northern hybridization analyses of RNA extracted from in vitro cultured tumour cells lines derived from different cancerous tissues. HELA2 (testisin) was detected in the HeLa ovarian carcinoma, the U937 lymphoma, and the melanoma cell line 253-3D. HELA2 (testisin) is also associated with cDNA libraries derived from tumours of the colon, pancreas, prostate and ovary  
20 (NCBI-EST Database). The presence of HELA2 (testisin) in tumours where it is not expressed normally indicates that it likely plays a role in tumourigenesis in several cell-types.

#### EXAMPLE 9

#### THE HELA2 (TESTISIN) GENE IS LOCATED 25 ON HUMAN CHROMOSOME 16p13.3

The genetic location of testisin was mapped to the short arm of chromosome 16 at 16p13.3 by fluorescence in-situ hybridization to normal metaphase chromosomes (Figure 13A). Screening of a chromosome 16 hybrid panel then sub-localised HELA2 (testisin) to the cosmid 406D6  
30 which has been mapped to this region (Sood, R. et al (1997) Genomics 42: 83-95; Doggett, N.A. et al. (1995) Nature 377 (Suppl.):335-365. The cosmid lies between the markers

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D16S246 and D16S468 and the gene is located just centromeric to D16S246 (Figure 13B). This region of the human genome is associated with high genetic instability and telomeric rearrangements underlie a variety of common human genetic disorders. Testisin is sandwiched between the human disease genes PKD1 (polycystic kidney disease) and tuberous sclerosis (TSC2) on the one side, and MEF (familial mediterranean fever) and Rubenstein-Taybi syndrome (RSTS) on the other side as diagrammed in Figure 13B.

### EXAMPLE 10

#### HELA2 (TESTISIN) mRNA AND PROTEIN EXPRESSION

#### IS ABSENT IN TESTICULAR GERM CELL TUMOURS

To determine whether HELA2 (testisin) may play a role in testicular tumourigenesis, HELA2 (testisin) mRNA expression in normal testes and testicular tumour tissue obtained from 4 patients diagnosed with seminoma were compared by Northern blot analysis. HELA2 (testisin) mRNA was detected in normal testes from all four patients but was not detectable in the corresponding tumours (Figure 14A). This data indicates a tumour suppressor role for HELA2 (testisin) in testicular germ cell tumours.

Expression of HELA2 (testisin) protein in testicular tissue was examined by immunohistochemistry. Paraffin-embedded tissue sections were fixed, treated, blocked, incubated with anti-peptide antibodies (1:10 dilution) and bound antibody detected with the Vectastain Universal Elite ABC kit (Vector Laboratories). Negative controls were performed in the absence of antibody. Strong staining of HELA2 (testisin) was detected in the germ cells of normal testis (N) but was absent in the adjacent tumour tissue (T) (for example, see Figure 14B), providing further evidence of a tumour suppressor role for HELA2 (testisin) in testicular germ cell tumours.

### EXAMPLE 11

#### GENOMIC ORGANISATION OF THE HELA2 (TESTISIN) GENE

The HELA2 (testisin) gene is further characterised by determination of its genomic

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organisation. Intron-extron boundaries and most of the DNA sequence of the HELA2 (testisin) gene was determined from cosmid DNA by DNA sequencing. A genomic map of HELA2 (testisin) is given in Figure 15. The intron/exon boundaries are highly conserved relative to prostasin, although the sizes of the introns show considerable variation. The genomic DNA sequence with introns in lower case and exons in upper case is shown in Figure 16 and in SEQ ID NO 25. DNA sequence analysis is being performed on RNA from tumour tissues to ascertain the predicted function of HELA2 (testisin) as a tumour suppressor.

#### EXAMPLE 12

#### 10 THE HELA2 (TESTISIN) SHORT AND LONG ISOFORMS ARE GENERATED BY ALTERNATIVE mRNA SPLICING

Two isoforms of HELA2 (testisin) were identified which differ by an insertion of 2 amino acids (Tyr-Ser) between the catalytic His and Asp residues. These constitute the long (L) and short (S) isoforms. At the DNA level there is a corresponding insertion of 6 nucleotides which generates a Sfc1 restriction enzyme site. PCR amplification from single strand cDNA generated from HeLa cell total RNA followed by DNA sequence analysis of the amplified product demonstrated that the two isoforms are generated through the use of two alternative mRNA splice sites. The DNA sequence for the intron and the flanking exons are shown in Figure 17. The resulting insertion of amino acids YS occurs 4 amino acids after the catalytic His residue of HELA2 (testisin). Preliminary molecular modelling shows the presence of this insertion is likely to alter the catalytic activity and/or specificity of HELA2 (testisin) for its substrates.

#### EXAMPLE 13

#### 25 MUTATION ANALYSIS-HELA2 (TESTISIN) AS A TUMOUR SUPPRESSOR

Intronic DNA sequence information generated above (see Example 11) is used to generate primers to amplify HELA2 (testisin) exons for SSCP analyses. Genomic DNA isolated from seminomas and corresponding normal testis as well as genomic DNA from wild-type and affected seminoma family members are analysed by SSCP for altered expression patterns indicative of genetic mutations. Evidence of genetic mutations are also being determined by

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DNA sequence analysis.

#### EXAMPLE 14

#### HOMOLOGUES OF HUMAN HELA2 (TESTISIN)

#### 5 ARE PRESENT IN OTHER SPECIES

Southern blot analysis of genomic DNA isolated from a range of species using a HELA2 (testisin) cDNA probe shows that homologues of HELA2 (testisin) are present in hamster, mouse, marmoset and monkey. The mouse homologue of HELA2 (testisin) was identified and  
10 obtained as an EST clone. The cDNA sequence and corresponding amino acid sequence of mouse HELA2 (testisin) was determined (Figure 18) and is given in SEQ ID NO 27. The mouse cDNA encodes a protein which contains the catalytic triad of His, Asp and Ser (circles) and 10 cysteine residues (small boxes), and an activation site (triangle) as found in HELA2 (testisin). The hydrophilicity plot shows the presence of a hydrophobic sequence at the carboxy  
15 terminus suggesting the presence of a putative membrane anchor. Comparison of the mouse and human sequences show 68.1% homology at the cDNA level and 69.1% homology at the amino acid level.

#### EXAMPLE 15

#### 20 HELA2 (TESTISIN) IS PART OF A CLUSTER OF HOMOLOGOUS GENES ON CHROMOSOME 16p13.3

Analysis of DNA sequences released to NCBI databases reveals the presence of homologues of HELA2 (testisin) in a cluster on Chromosome 16p13.3. Figure 19 shows the positions of  
25 these genes, designated SP001LA, SP002LA, SP003LA, and SP004LA, relative to HELA2 (testisin) and the respective cosmids (Sood, R. et al (1997) Genomics 42: 83-95) in which they are located. Figure 20A, 20B and 20C show the partial cDNA and deduced amino acid sequences of SP001LA, SP002LA, and SP003LA respectively. Each cDNA encodes a protein which contains the catalytic triad of His, Asp and Ser (circles) and 10 cysteine residues (small  
30 boxes), and an activation site (triangle) as found in HELA2 (testisin). Comparisons of the cDNA and amino acid sequences from the heavy chain region through to the poly A tail gives

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the % identity with HELA2 (testisin) as follows:

	cDNA	Protein
SP001LA	34.8%	47.3%
5 SP002LA	41.0%	47.1%
SP003LA	40.3%	51.3%

Each of the serine proteinases encoded by these genes show that they have carboxy terminal extensions, and SP002LA is the only one with a hydrophobic carboxy terminal tail indicative of a membrane anchored protein. Identification of an expressed sequence tag (EST) from a human testis cDNA library demonstrates that this gene is expressed in the testis, like HELA2 (testisin). The location of this serine proteinase cluster on chromosome 16p13.3 flanking HELA2 (testisin) suggests that these serine proteinases are also involved, like HELA2(testisin), in sperm maturation and development. Thus they may constitute a proteolytic cascade which is essential for these processes. Loss or mutation of these genes may lead to testicular germ cell tumours and to other testicular abnormalities, such as infertility.

### EXAMPLE 16

#### ATC2 SERINE PROTEINASE

20

ATC2 was isolated from the cDNA of PAI-2 expressing HeLa cells following treatment with TNF and cycloheximide. A partial DNA sequence for ATC2 cDNA has been obtained which encompasses the sequence encoding the serine proteinase catalytic region. Additional clones extending to both 5' and 3' directions have been obtained. The available nucleic acid sequence of ATC2 cDNA and its deduced amino acid sequence shows that it is a member of the serine proteinase family with homology to hepsin, prostasin, and acrosin. It thus belongs to the same family as HELA2. The catalytic region includes the His, Asp and Ser conserved motifs. Preliminary Northern blot experiments have failed to detect ATC2 mRNA in total RNA isolated from resting HeLa cells, indicating it is not expressed in abundance in these cells, which may therefore be tightly regulated. As ATC2 was isolated from cells following treatment with TNF and cycloheximide, its expression may be induced by these agents in HeLa cells. These data

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have potential significance for a role for ATC2 in apoptosis and cell death. ATC2 may be intracellular, extracellular or found on the cell surface and is likely to be involved in regulating cell functions. Thus ATC2 may have potential significance in the treatment of cancer and diseases involving dysregulation of cell growth and survival. The nucleotide and corresponding amino acid sequence of ATC2 is shown in SEQ ID NOs: 7 and 8, respectively.

### EXAMPLE 16

#### BCON3

10

The deduced amino acid sequence of BCON3 (SEQ ID NO:10) reveals that it is novel. At both the DNA and protein level, BCON3 shows homology to members of the kinase family of proteins. Although it cannot be classified as a member of any particular sub-family of kinases, alignments of the BCON3 protein with the conserved domains of thymidine kinases and tyrosine and serine/threonine protein kinases indicates possible ATP/GTP binding and phosphate transfer regions. Thus, it may be the first member of a new family of kinases. Analysis of the translation product using hydrophobicity plots and the Prosite protein analysis algorithms indicates BCON3 may lack an N-terminal signal sequence (that is, it is likely to encode an intracellular protein) and it possesses a nuclear localization signal. BCON3 mRNA is approximately 2300 nucleotides in length. cDNA sequence (SEQ ID NO:9) has been obtained covering about 95% of the transcript and including the 3' poly A tail. BCON3 mRNA is expressed in most normal tissues as demonstrated by dot blot analysis of 50 normal tissue specimens (standardised to 8 different housekeeping genes) (Clontech). (Figure 9). Analysis of BCON3 mRNA expression using a multiple tissue Northern blot displaying polyA+ mRNA from 16 different normal tissues (Clontech) shows that BCON3 is expressed in most tissues (Figure 10B). Expression by in vitro transcription/translation expression using a partial BCON3 cDNA fragment shows BCON3 encodes a protein. Two major transcription/translation products are detected, one of 51kDa, the size predicted from the open reading frame, and a second product of about 43kDa, which may represent a partial translation product (Figure 21).

25  
30

Those skilled in the art will appreciate that the invention described herein is susceptible to



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variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or  
5 features.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT

(US only): ANTALIS Toni Marie and HOOPER John David  
(Other than US): AMRAD OPERATIONS PTY LTD

## (ii) TITLE OF INVENTION: NOVEL MOLECULES

## (iii) NUMBER OF SEQUENCES: 24

## (iv) CORRESPONDENCE ADDRESS:

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(D) STATE: VICTORIA  
(E) COUNTRY: AUSTRALIA  
(F) ZIP: 3000

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: International PCT Application  
(B) FILING DATE: 13-FEB-1998  
(C) CLASSIFICATION:

## (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PO5101/97  
(B) FILING DATE: 13-FEB-1997  
(C) CLASSIFICATION:

## (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PP0422/97  
(B) FILING DATE: 18-NOV-1997  
(C) CLASSIFICATION:

## (viii) ATTORNEY/AGENT INFORMATION:

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(C) REFERENCE/DOCKET NUMBER: EJH/AF

- 57 -

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(B) TELEFAX: +61 3 9254 2770

(C) TELEX: AA 31787

- 58 -

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 32 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ACAGAATTCT GGGTIGTIAC IGCIGCICAY TG

32

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1094 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACAGAATTCA XIGGICCICC IC/GT/AXTCICC

29

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1094 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 17..965

- 59 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGCGGGAGAG GAGGCC ATG GGC GCG CGC GGG GCG CTG CTG CTG GCG CTG	49
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1 5 10	
CTG CTG GCT CGG GCT GGA CTC AGG AAG CCG GAG TCG CAG GAG GCG GCG	97
Leu Leu Ala Arg Ala Gly Leu Arg Lys Pro Glu Ser Gln Glu Ala Ala	
15 20 25	
CCG TTA TCA GGA CCA TGC GGC CGA CGG GTC ATC ACG TCG CGC ATC GTG	145
Pro Leu Ser Gly Pro Cys Gly Arg Arg Val Ile Thr Ser Arg Ile Val	
30 35 40	
GGT GGA GAG GAC GCC GAA CTC GGG CGT TGG CCG TGG CAG GGG AGC CTG	193
Gly Gly Glu Asp Ala Glu Leu Gly Arg Trp Pro Trp Gln Gly Ser Leu	
45 50 55	
CGC CTG TGG GAT TCC CAC GTA TGC GGA GTG AGC CTG CTC AGC CAC CGC	241
Arg Leu Trp Asp Ser His Val Cys Gly Val Ser Leu Leu Ser His Arg	
60 65 70 75	
TGG GCA CTC ACG GCG GCG CAC TGC TTT GAA ACT GAC CTT AGT GAT CCC	289
Trp Ala Leu Thr Ala Ala His Cys Phe Glu Thr Asp Leu Ser Asp Pro	
80 85 90	
TCC GGG TGG ATG GTC CAG TTT GGC CAG CTG ACT TCC ATG CCA TCC TTC	337
Ser Gly Trp Met Val Gln Phe Gly Gln Leu Thr Ser Met Pro Ser Phe	
95 100 105	
TGG AGC CTG CAG GCC TAC TAC ACC CGT TAC TTC GTA TCG AAT ATC TAT	385
Trp Ser Leu Gln Ala Tyr Tyr Thr Arg Tyr Phe Val Ser Asn Ile Tyr	
110 115 120	
CTG AGC CCT CGC TAC CTG GGG AAT TCA CCC TAT GAC ATT GCC TTG GTG	433
Leu Ser Pro Arg Tyr Leu Gly Asn Ser Pro Tyr Asp Ile Ala Leu Val	
125 130 135	
AAG CTG TCT GCA CCT GTC ACC TAC ACT AAA CAC ATC CAG CCC ATC TGT	481
Lys Leu Ser Ala Pro Val Thr Tyr Thr Lys His Ile Gln Pro Ile Cys	
140 145 150 155	
CTC CAG GCC TCC ACA TTT GAG TTT GAG AAC CGG ACA GAC TGC TGG GTG	529
Leu Gln Ala Ser Thr Phe Glu Phe Glu Asn Arg Thr Asp Cys Trp Val	
160 165 170	

- 60 -

ACT GGC TGG GGG TAC ATC AAA GAG GAT GAG GCA CTG CCA TCT CCC CAC	577
Thr Gly Trp Gly Tyr Ile Lys Glu Asp Glu Ala Leu Pro Ser Pro His	
175 180 185	
ACC CTC CAG GAA GTT CAG GTC GCC ATC ATA AAC AAC TCT ATG TGC AAC	625
Thr Leu Gln Glu Val Gln Val Ala Ile Ile Asn Asn Ser Met Cys Asn	
190 195 200	
CAC CTC TTC CTC AAG TAC AGT TTC CGC AAG GAC ATC TTT GGA GAC ATG	673
His Leu Phe Leu Lys Tyr Ser Phe Arg Lys Asp Ile Phe Gly Asp Met	
205 210 215	
GTT TGT GCT GGC AAT GCC CAA GGC GGG AAG GAT GCC TGC TTC GGT GAC	721
Val Cys Ala Gly Asn Ala Gln Gly Gly Lys Asp Ala Cys Phe Gly Asp	
220 225 230 235	
TCA GGT GGA CCC TTG GCC TGT AAC AAG GAT GGA CTG TGG TAT CAG ATT	769
Ser Gly Gly Pro Leu Ala Cys Asn Lys Asp Gly Leu Trp Tyr Gln Ile	
240 245 250	
GGA GTC GTG AGC TGG GGA GTG GGC TGT GGT CGG CCC AAT CGG CCC GGT	817
Gly Val Val Ser Trp Gly Val Gly Cys Gly Arg Pro Asn Arg Pro Gly	
255 260 265	
GTC TAC ACC AAT ATC AGC CAC CAC TTT GAG TGG ATC CAG AAG CTG ATG	865
Val Tyr Thr Asn Ile Ser His His Phe Glu Trp Ile Gln Lys Leu Met	
270 275 280	
GCC CAG AGT GGC ATG TCC CAG CCA GAC CCC TCC TGG CCG CTA CTC TTT	913
Ala Gln Ser Gly Met Ser Gln Pro Asp Pro Ser Trp Pro Leu Leu Phe	
285 290 295	
TTC CCT CTT CTC TGG GCT CTC CCA CTC CTG GGG CCG GTC TGA	961
Phe Pro Leu Leu Trp Ala Leu Pro Leu Leu Gly Pro Val *	
300 305 310	
GCCTACCTGA GCCCATGCAG CCTGGGGCCA CTGCCAAGTC AGGCCCTGGT TCTCTTCTGT	1015
CTTGTTTGGT AATAAACACA TTCCAGTTGA TGCCTTGCAG GGCATTTTTC AAAAAAAAAA	1075
AAAAAAAAAA AAAAAAAAAA	1094

(2) INFORMATION FOR SEQ ID NO:4:

- 61 -

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 313 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Gly Ala Arg Gly Ala Leu Leu Leu Ala Leu Leu Leu Ala Arg Ala
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      20               25               30

Cys Gly Arg Arg Val Ile Thr Ser Arg Ile Val Gly Gly Glu Asp Ala
      35               40               45

Glu Leu Gly Arg Trp Pro Trp Gln Gly Ser Leu Arg Leu Trp Asp Ser
      50               55               60

His Val Cys Gly Val Ser Leu Leu Ser His Arg Trp Ala Leu Thr Ala
      65               70               75               80

Ala His Cys Phe Glu Thr Asp Leu Ser Asp Pro Ser Gly Trp Met Val
      85               90               95

Gln Phe Gly Gln Leu Thr Ser Met Pro Ser Phe Trp Ser Leu Gln Ala
      100              105              110

Tyr Tyr Thr Arg Tyr Phe Val Ser Asn Ile Tyr Leu Ser Pro Arg Tyr
      115              120              125

Leu Gly Asn Ser Pro Tyr Asp Ile Ala Leu Val Lys Leu Ser Ala Pro
      130              135              140

Val Thr Tyr Thr Lys His Ile Gln Pro Ile Cys Leu Gln Ala Ser Thr
      145              150              155              160

Phe Glu Phe Glu Asn Arg Thr Asp Cys Trp Val Thr Gly Trp Gly Tyr
      165              170              175

Ile Lys Glu Asp Glu Ala Leu Pro Ser Pro His Thr Leu Gln Glu Val
      180              185              190

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Gln Val Ala Ile Ile Asn Asn Ser Met Cys Asn His Leu Phe Leu Lys  
 195 200 205  
 Tyr Ser Phe Arg Lys Asp Ile Phe Gly Asp Met Val Cys Ala Gly Asn  
 210 215 220  
 Ala Gln Gly Gly Lys Asp Ala Cys Phe Gly Asp Ser Gly Gly Pro Leu  
 225 230 235 240  
 Ala Cys Asn Lys Asp Gly Leu Trp Tyr Gln Ile Gly Val Val Ser Trp  
 245 250 255  
 Gly Val Gly Cys Gly Arg Pro Asn Arg Pro Gly Val Tyr Thr Asn Ile  
 260 265 270  
 Ser His His Phe Glu Trp Ile Gln Lys Leu Met Ala Gln Ser Gly Met  
 275 280 285  
 Ser Gln Pro Asp Pro Ser Trp Pro Leu Leu Phe Phe Pro Leu Leu Trp  
 290 295 300  
 Ala Leu Pro Leu Leu Gly Pro Val \*  
 305 310

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1100 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 17..961

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGCGGGAGAG GAGGCC ATG GGC GCG CGC GGG GCG CTG CTG CTG GCG CTG  
 Met Gly Ala Arg Gly Ala Leu Leu Leu Ala Leu

49



- 63 -

	1	5	10	
CTG CTG GCT CGG GCT GGA CTC AGG AAG CCG GAG TCG CAG GAG GCG GCG				97
Leu Leu Ala Arg Ala Gly Leu Arg Lys Pro Glu Ser Gln Glu Ala Ala				
	15	20	25	
CCG TTA TCA GGA CCA TGC GGC CGA CGG GTC ATC ACG TCG CGC ATC GTG				145
Pro Leu Ser Gly Pro Cys Gly Arg Arg Val Ile Thr Ser Arg Ile Val				
	30	35	40	
GGT GGA GAG GAC GCC GAA CTC GGG CGT TGG CCG TGG CAG GGG AGC CTG				193
Gly Gly Glu Asp Ala Glu Leu Gly Arg Trp Pro Trp Gln Gly Ser Leu				
	45	50	55	
CGC CTG TGG GAT TCC CAC GTA TGC GGA GTG AGC CTG CTC AGC CAC CGC				241
Arg Leu Trp Asp Ser His Val Cys Gly Val Ser Leu Leu Ser His Arg				
	60	65	70	75
TGG GCA CTC ACG GCG GCG CAC TGC TTT GAA ACC TAT AGT GAC CTT AGT				289
Trp Ala Leu Thr Ala Ala His Cys Phe Glu Thr Tyr Ser Asp Leu Ser				
	80	85	90	
GAT CCC TCC GGG TGG ATG GTC CAG TTT GGC CAG CTG ACT TCC ATG CCA				337
Asp Pro Ser Gly Trp Met Val Gln Phe Gly Gln Leu Thr Ser Met Pro				
	95	100	105	
TCC TTC TGG AGC CTG CAG GCC TAC TAC ACC CGT TAC TTC GTA TCG AAT				385
Ser Phe Trp Ser Leu Gln Ala Tyr Tyr Thr Arg Tyr Phe Val Ser Asn				
	110	115	120	
ATC TAT CTG AGC CCT CGC TAC CTG GGG AAT TCA CCC TAT GAC ATT GCC				433
Ile Tyr Leu Ser Pro Arg Tyr Leu Gly Asn Ser Pro Tyr Asp Ile Ala				
	125	130	135	
TTG GTG AAG CTG TCT GCA CCT GTC ACC TAC ACT AAA CAC ATC CAG CCC				481
Leu Val Lys Leu Ser Ala Pro Val Thr Tyr Thr Lys His Ile Gln Pro				
	140	145	150	155
ATC TGT CTC CAG GCC TCC ACA TTT GAG TTT GAG AAC CGG ACA GAC TGC				529
Ile Cys Leu Gln Ala Ser Thr Phe Glu Phe Glu Asn Arg Thr Asp Cys				
	160	165	170	
TGG GTG ACT GGC TGG GGG TAC ATC AAA GAG GAT GAG GCA CTG CCA TCT				577
Trp Val Thr Gly Trp Gly Tyr Ile Lys Glu Asp Glu Ala Leu Pro Ser				
	175	180	185	

- 64 -

CCC CAC ACC CTC CAG GAA GTT CAG GTC GCC ATC ATA AAC AAC TCT ATG	625
Pro His Thr Leu Gln Glu Val Gln Val Ala Ile Ile Asn Asn Ser Met	
190 195 200	
TGC AAC CAC CTC TTC CTC AAG TAC AGT TTC CGC AAG GAC ATC TTT GGA	673
Cys Asn His Leu Phe Leu Lys Tyr Ser Phe Arg Lys Asp Ile Phe Gly	
205 210 215	
GAC ATG GTT TGT GCT GGC AAT GCC CAA GGC GGG AAG GAT GCC TGC TTC	721
Asp Met Val Cys Ala Gly Asn Ala Gln Gly Gly Lys Asp Ala Cys Phe	
220 225 230 235	
GGT GAC TCA GGT GGA CCC TTG GCC TGT AAC AAG GAT GGA CTG TGG TAT	769
Gly Asp Ser Gly Gly Pro Leu Ala Cys Asn Lys Asp Gly Leu Trp Tyr	
240 245 250	
CAG ATT GGA GTC GTG AGC TGG GGA GTG GGC TGT GGT CGG CCC AAT CGG	817
Gln Ile Gly Val Val Ser Trp Gly Val Gly Cys Gly Arg Pro Asn Arg	
255 260 265	
CCC GGT GTC TAC ACC AAT ATC AGC CAC CAC TTT GAG TGG ATC CAG AAG	865
Pro Gly Val Tyr Thr Asn Ile Ser His His Phe Glu Trp Ile Gln Lys	
270 275 280	
CTG ATG GCC CAG AGT GGC ATG TCC CAG CCA GAC CCC TCC TGG CCG CTA	913
Leu Met Ala Gln Ser Gly Met Ser Gln Pro Asp Pro Ser Trp Pro Leu	
285 290 295	
CTC TTT TTC CCT CTT CTC TGG GCT CTC CCA CTC CTG GGG CCG GTC TGAGCCTACC	
968	
Leu Phe Phe Pro Leu Leu Trp Ala Leu Pro Leu Leu Gly Pro Val	
300 305 310 315	
TGAGCCCATG CAGCCTGGGG CCACTGCCAA GTCAGGCCCT GGTCTCTTC TGTCTTGTTT	1028
GGTAATAAAC ACATTCCAGT TGATGCCTTG CAGGGCATT TTTCAAAAAA AAAAAA	1088
AAAAA AA	1100

(2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 314 amino acids  
 (B) TYPE: amino acid

- 65 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Gly Ala Arg Gly Ala Leu Leu Leu Ala Leu Leu Leu Ala Arg Ala
 1             5             10             15

Gly Leu Arg Lys Pro Glu Ser Gln Glu Ala Ala Pro Leu Ser Gly Pro
      20             25             30

Cys Gly Arg Arg Val Ile Thr Ser Arg Ile Val Gly Gly Glu Asp Ala
      35             40             45

Glu Leu Gly Arg Trp Pro Trp Gln Gly Ser Leu Arg Leu Trp Asp Ser
      50             55             60

His Val Cys Gly Val Ser Leu Leu Ser His Arg Trp Ala Leu Thr Ala
      65             70             75             80

Ala His Cys Phe Glu Thr Tyr Ser Asp Leu Ser Asp Pro Ser Gly Trp
      85             90             95

Met Val Gln Phe Gly Gln Leu Thr Ser Met Pro Ser Phe Trp Ser Leu
      100            105            110

Gln Ala Tyr Tyr Thr Arg Tyr Phe Val Ser Asn Ile Tyr Leu Ser Pro
      115            120            125

Arg Tyr Leu Gly Asn Ser Pro Tyr Asp Ile Ala Leu Val Lys Leu Ser
      130            135            140

Ala Pro Val Thr Tyr Thr Lys His Ile Gln Pro Ile Cys Leu Gln Ala
      145            150            155            160

Ser Thr Phe Glu Phe Glu Asn Arg Thr Asp Cys Trp Val Thr Gly Trp
      165            170            175

Gly Tyr Ile Lys Glu Asp Glu Ala Leu Pro Ser Pro His Thr Leu Gln
      180            185            190

Glu Val Gln Val Ala Ile Ile Asn Asn Ser Met Cys Asn His Leu Phe
      195            200            205

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Leu Lys Tyr Ser Phe Arg Lys Asp Ile Phe Gly Asp Met Val Cys Ala  
 210 215 220  
 Gly Asn Ala Gln Gly Gly Lys Asp Ala Cys Phe Gly Asp Ser Gly Gly  
 225 230 235 240  
 Pro Leu Ala Cys Asn Lys Asp Gly Leu Trp Tyr Gln Ile Gly Val Val  
 245 250 255  
 Ser Trp Gly Val Gly Cys Gly Arg Pro Asn Arg Pro Gly Val Tyr Thr  
 260 265 270  
 Asn Ile Ser His His Phe Glu Trp Ile Gln Lys Leu Met Ala Gln Ser  
 275 280 285  
 Gly Met Ser Gln Pro Asp Pro Ser Trp Pro Leu Leu Phe Phe Pro Leu  
 290 295 300  
 Leu Trp Ala Leu Pro Leu Leu Gly Pro Val  
 305 310

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 799 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 24..799

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AGTTCAGATG AATGGGACTG TGA GAA CCA TCT GTG ACC AAA TTG ATA CAG 50  
 Glu Pro Ser Val Thr Lys Leu Ile Gln  
 1 5  
 GAA CAG GAG AAA GAG CCG CGG TGG CTG ACA TTA CAC TCC AAC TGG GAG 98



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190	195	200	
GGC AAT AAA ATG CCA TTT AAG CTG CAA GAG GGA GAG GTC CGC ATT ATT			674
Gly Asn Lys Met Pro Phe Lys Leu Gln Glu Gly Glu Val Arg Ile Ile			
205	210	215	
TCT CTG GAA CAT TGT CAG TCC TAC TTT GAC ATG AAG ACC ATC ACC ACT			722
Ser Leu Glu His Cys Gln Ser Tyr Phe Asp Met Lys Thr Ile Thr Thr			
220	225	230	
CGG ATG ATA TGT GCT GGC TAT GAG TCT GGC ACA GTT GAT TCA TGC ATG			770
Arg Met Ile Cys Ala Gly Tyr Glu Ser Gly Thr Val Asp Ser Cys Met			
235	240	245	
GGT GAC TGG GGC GGT CCG TTG AAT TCT GT			799
Gly Asp Trp Gly Gly Pro Leu Asn Ser			
250	255		

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 258 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Glu Pro Ser Val Thr Lys Leu Ile Gln Glu Gln Glu Lys Glu Pro Arg			
1	5	10	15
Trp Leu Thr Leu His Ser Asn Trp Glu Ser Leu Asn Gly Thr Thr Leu			
20	25	30	
His Glu Leu Val Val Asn Gly Gln Ser Cys Glu Ser Arg Ser Lys Ile			
35	40	45	
Ser Leu Leu Cys Thr Lys Gln Asp Cys Gly Arg Arg Pro Ala Ala Arg			
50	55	60	
Met Asn Lys Arg Ile Leu Gly Gly Arg Thr Ser Arg Pro Gly Arg Trp			
65	70	75	80

Pro Trp Gln Cys Ser Leu Gln Ser Glu Pro Ser Gly His Ile Cys Gly  
85 90 95

Cys Val Leu Ile Ala Lys Lys Trp Val Val Thr Val Ala His Cys Phe  
100 105 110

Glu Gly Arg Glu Asn Ala Ala Val Trp Lys Val Val Leu Gly Ile Asn  
115 120 125

Asn Leu Asp His Pro Ser Val Phe Met Gln Thr Arg Phe Val Arg Thr  
130 135 140

Ile Ile Leu His Pro Arg Tyr Ser Arg Ala Val Val Asp Tyr Asp Ile  
145                   150                   155                   160

Ser Ile Val Glu Leu Ser Glu Asp Ile Ser Glu Thr Gly Tyr Val Arg  
165 170 175

Pro Val Cys Leu Pro Asn Pro Glu Gln Trp Leu Glu Pro Asp Thr Tyr  
180 185 190

Cys Tyr Ile Thr Gly Trp Gly His Met Gly Asn Lys Met Pro Phe Lys  
195 200 205

Leu Gln Glu Gly Glu Val Arg Ile Ile Ser Leu Glu His Cys Gln Ser  
210 215 220

Tyr Phe Asp Met Lys Thr Ile Thr Thr Arg Met Ile Cys Ala Gly Tyr  
225 230 235 240

Glu Ser Gly Thr Val Asp Ser Cys Met Gly Asp Trp Gly Gly Pro Leu  
245 250 255

Asn Ser

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2241 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 166..1773

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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ATTTAATACG ACTCACTATA GGGAATTTGG CCCTCGAGGA AGAATTCGGC ACGAGGCTGC      60
GGCGCACTGT GAGGGAGTCG CTGTGATCCG GGGCCCCGAA CCCGACTGGA GCTGAAGCGC      120
AGGCTGCGGG GCGCGGAGTC GGGAGGCCTG AGTGTTCTTT CCAGC ATG TCG GAG      174
                                   Met Ser Glu
                                   1

GGG GAG TCC CAG ACA GTA CTT AGC AGT GGC TCA GAC CCA AAG GTA GAA      222
Gly Glu Ser Gln Thr Val Leu Ser Ser Gly Ser Asp Pro Lys Val Glu
      5              10              15

TCT TCA TCT TCA GCT CCT GGC CTG ACA TCA GTG TCA CCT CCT GTG ACC      270
Ser Ser Ser Ser Ala Pro Gly Leu Thr Ser Val Ser Pro Pro Val Thr
      20              25              30              35

TCC ACA ACC TCA GCT GCT TCC CCA GAG GAA GAA GAA GAA AGT GAA GAT      318
Ser Thr Thr Ser Ala Ala Ser Pro Glu Glu Glu Glu Glu Ser Glu Asp
              40              45              50

GAG TCT GAG ATT TTG GAA GAG TCG CCC TGT GGG CGC TGG CAG AAG AGG      366
Glu Ser Glu Ile Leu Glu Glu Ser Pro Cys Gly Arg Trp Gln Lys Arg
              55              60              65

CGA GAA GAG GTG AAT CAA CGG AAT GTA CCA GGT ATT GAC AGT GCA TAC      414
Arg Glu Glu Val Asn Gln Arg Asn Val Pro Gly Ile Asp Ser Ala Tyr
              70              75              80

CTG GCC ATG GAT ACA GAG GAA GGT GTA GAG GTT GTG TGG AAT GAG GTA      462
Leu Ala Met Asp Thr Glu Glu Gly Val Glu Val Val Trp Asn Glu Val
      85              90              95

CAG TTC TCT GAA CGC AAG AAC TAC AAG CTG CAG GAG GAA AAG GTT TGT      510
Gln Phe Ser Glu Arg Lys Asn Tyr Lys Leu Gln Glu Glu Lys Val Cys
      100              105              110              115

GCT GTG TTT GAT AAT TTG ATT CAA TTG GAG CAT CTT AAC ATT GTT AAG      558

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Ala Val Phe Asp Asn Leu Ile Gln Leu Glu His Leu Asn Ile Val Lys	
120 125 130	
TTT CAC AAA TAT TGG GCT GAC ATT AAA GAG AAC AAG GCC AGG GTC ATT	606
Phe His Lys Tyr Trp Ala Asp Ile Lys Glu Asn Lys Ala Arg Val Ile	
135 140 145	
TTT ATC ACA GGA TAC ATG TCA TCT GGG AGT CTG AAG CAA TTT CTG AAG	654
Phe Ile Thr Gly Tyr Met Ser Ser Gly Ser Leu Lys Gln Phe Leu Lys	
150 155 160	
AAG ACC CAA AAG AAC CAC CAG ACG ATG AAT GAA AAG GCA TGG AAG CGT	702
Lys Thr Gln Lys Asn His Gln Thr Met Asn Glu Lys Ala Trp Lys Arg	
165 170 175	
TGG TGC ACA CAA ATC CTC TCT GCC CTA AGC TAC CTG CAC TCC TGT GAC	750
Trp Cys Thr Gln Ile Leu Ser Ala Leu Ser Tyr Leu His Ser Cys Asp	
180 185 190 195	
CCC CCC ATC ATC CAT GGG AAC CTG ACC TGT GAC ACC ATC TTC ATC CAG	798
Pro Pro Ile Ile His Gly Asn Leu Thr Cys Asp Thr Ile Phe Ile Gln	
200 205 210	
CAC AAC GGA CTC ATC AAG ATT GGC TCT GTG GCT CCT GAC ACT ATC AAC	846
His Asn Gly Leu Ile Lys Ile Gly Ser Val Ala Pro Asp Thr Ile Asn	
215 220 225	
AAT CAT GTG AAG ACT TGT CGA GAA GAG CAG AAG AAT CTA CAC TTC TTT	894
Asn His Val Lys Thr Cys Arg Glu Glu Gln Lys Asn Leu His Phe Phe	
230 235 240	
GCA CCA GAC TAT GGA GAA GTC ACT AAT GTG ACA ACA GCA GTG GAC ATC	942
Ala Pro Glu Tyr Gly Glu Val Thr Asn Val Thr Thr Ala Val Asp Ile	
245 250 255	
TAC TCC TTT GGC ATG TGT GCA CTG GGG ATG GCA GTG CTG GAG ATT CAG	990
Tyr Ser Phe Gly Met Cys Ala Leu Gly Met Ala Val Leu Glu Ile Gln	
260 265 270 275	
GGC AAT GGA GAG TCC TCA TAT GTG CCA CAG GAA GCC ATC AGC AGT GCC	1038
Gly Asn Gly Glu Ser Ser Tyr Val Pro Gln Glu Ala Ile Ser Ser Ala	
280 285 290	
ATC CAG CTT CTA GAA GAC CCA TTA CAG AGG GAG TTC ATT CAA AAG TGC	1086
Ile Gln Leu Leu Glu Asp Pro Leu Gln Arg Glu Phe Ile Gln Lys Cys	

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295	300	305	
CTG CAG TCT GAG CCT GCT CGC AGA CCA ACA GCC AGA GAA CTT CTG TTC			1134
Leu Gln Ser Glu Pro Ala Arg Arg Pro Thr Ala Arg Glu Leu Leu Phe			
310	315	320	
CAC CCA GCA TTG TTT GAA GTG CCC TCG CTC AAA CTC CTT GCG GCC CAC			1182
His Pro Ala Leu Phe Glu Val Pro Ser Leu Lys Leu Leu Ala Ala His			
325	330	335	
TGC ATT GTG GGA CAC CAA CAC ATG ATC CCA GAG AAC GCT CTA GAG GAG			1230
Cys Ile Val Gly His Gln His Met Ile Pro Glu Asn Ala Leu Glu Glu			
340	345	350	355
ATC ACC AAA AAC ATG GAT ACT AGT GCC GTA CTG GCT GAA ATC CCT GCA			1278
Ile Thr Lys Asn Met Asp Thr Ser Ala Val Leu Ala Glu Ile Pro Ala			
360	365	370	
GGA CCA GGA AGA GAA CCA GTT CAG ACT TTG TAC TCT CAG TCA CCA GCT			1326
Gly Pro Gly Arg Glu Pro Val Gln Thr Leu Tyr Ser Gln Ser Pro Ala			
375	380	385	
CTG GAA TTA GAT AAA TTC CTT GAA GAT GTC AGG AAT GGG ATC TAT CCT			1374
Leu Glu Leu Asp Lys Phe Leu Glu Asp Val Arg Asn Gly Ile Tyr Pro			
390	395	400	
CTG ACA GCC TTT GGG CTG CCT CGG CCC CAG CAG CCA CAG CAG GAG GAG			1422
Leu Thr Ala Phe Gly Leu Pro Arg Pro Gln Gln Pro Gln Gln Glu Glu			
405	410	415	
GTG ACA TCA CCT GTC GTG CCC CCC TCT GTC AAG ACT CCG ACA CCT GAA			1470
Val Thr Ser Pro Val Val Pro Pro Ser Val Lys Thr Pro Thr Pro Glu			
420	425	430	435
CCA GCT GAG GTG GAG ACT CGC AAG GTG GTG CTG ATG CAG TGC AAC ATT			1518
Pro Ala Glu Val Glu Thr Arg Lys Val Val Leu Met Gln Cys Asn Ile			
440	445	450	
GAG TCG GTG GAG GAG GGA GTC AAA CAC CAC CTG ACA CTT CTG CTG AAG			1566
Glu Ser Val Glu Glu Gly Val Lys His His Leu Thr Leu Leu Leu Lys			
455	460	465	
TTG GAG GAC AAA CTG AAC CGG CAC CTG AGC TGT GAC CTG ATG CCA AAT			1614
Leu Glu Asp Lys Leu Asn Arg His Leu Ser Cys Asp Leu Met Pro Asn			
470	475	480	

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GAG AAT ATC CCC GAG TTG GCG GCT GAG CTG GTG CAG CTG GGC TTC ATT	1662
Glu Asn Ile Pro Glu Leu Ala Ala Glu Leu Val Gln Leu Gly Phe Ile	
485 490 495	
AGT GAG GCT GAC CAG AGC CGG TTG ACT TCT CTG CTA GAA GAG ACC TTG	1710
Ser Glu Ala Asp Gln Ser Arg Leu Thr Ser Leu Leu Glu Glu Thr Leu	
500 505 510 515	
AAC AAG TTC AAT TTT GCC AGG AAC AGT ACC CTC AAC TCA GCC GCT GTC	1758
Asn Lys Phe Asn Phe Ala Arg Asn Ser Thr Leu Asn Ser Ala Ala Val	
520 525 530	
ACC GTC TCC TCT TAGAGCTCAC TCGGGCCAGG CCCTGATCTG CGCTGTGGCT	1810
Thr Val Ser Ser	
535	
GTCCCTGGAC GTGCTGCAGC CCTCCTGTCC CTTCCCCCA GTCAGTATTA CCCTGTGAAG	1870
CCCCCTCCCT CCTTTATTAT TCAGGAGGGC TGGGGGGGCT CCCTGGTTCT GAGCATCATC	1930
CTTTCCCCTC CCCTCTCTTC CTCCCCTCTG CACTTTGTTT ACTTGTTTTG CACAGACGTG	1990
GGCCTGGGCC TTCTCAGCAG CCGCCTTCTA GTTGGGGGCT AGTCGCTGAT CTGCCGGCTC	2050
CCGCCCAGCC TGTGTGGAAA GGAGGCCAC GGGCACTAGG GGAGCCGAAT TCTACAATCC	2110
CGCTGGGGCG GCCGGGGCGG GAGAGAAAGG TGGTGCTGCA GTGGTGCCCC TGGGGGGCCA	2170
TTCGATTGCG CTCAGTTGCT GCTGTAATAA AAGTCTACTT TTTGCTAAAA AAAAAAAAAA	2230
AAAAAAAAA A	2241

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 535 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ser Glu Gly Glu Ser Gln Thr Val Leu Ser Ser Gly Ser Asp Pro

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1	5	10	15
Lys Val Glu Ser Ser Ser Ser Ala Pro Gly Leu Thr Ser Val Ser Pro			
20	25	30	
Pro Val Thr Ser Thr Thr Ser Ala Ala Ser Pro Glu Glu Glu Glu Glu			
35	40	45	
Ser Glu Asp Glu Ser Glu Ile Leu Glu Glu Ser Pro Cys Gly Arg Trp			
50	55	60	
Gln Lys Arg Arg Glu Glu Val Asn Gln Arg Asn Val Pro Gly Ile Asp			
65	70	75	80
Ser Ala Tyr Leu Ala Met Asp Thr Glu Glu Gly Val Glu Val Val Trp			
85	90	95	
Asn Glu Val Gln Phe Ser Glu Arg Lys Asn Tyr Lys Leu Gln Glu Glu			
100	105	110	
Lys Val Cys Ala Val Phe Asp Asn Leu Ile Gln Leu Glu His Leu Asn			
115	120	125	
Ile Val Lys Phe His Lys Tyr Trp Ala Asp Ile Lys Glu Asn Lys Ala			
130	135	140	
Arg Val Ile Phe Ile Thr Gly Tyr Met Ser Ser Gly Ser Leu Lys Gln			
145	150	155	160
Phe Leu Lys Lys Thr Gln Lys Asn His Gln Thr Met Asn Glu Lys Ala			
165	170	175	
Trp Lys Arg Trp Cys Thr Gln Ile Leu Ser Ala Leu Ser Tyr Leu His			
180	185	190	
Ser Cys Asp Pro Pro Ile Ile His Gly Asn Leu Thr Cys Asp Thr Ile			
195	200	205	
Phe Ile Gln His Asn Gly Leu Ile Lys Ile Gly Ser Val Ala Pro Asp			
210	215	220	
Thr Ile Asn Asn His Val Lys Thr Cys Arg Glu Glu Gln Lys Asn Leu			
225	230	235	240
His Phe Phe Ala Pro Glu Tyr Gly Glu Val Thr Asn Val Thr Thr Ala			

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245	250	255
Val Asp Ile Tyr Ser Phe Gly Met Cys Ala Leu Gly Met Ala Val Leu		
260	265	270
Glu Ile Gln Gly Asn Gly Glu Ser Ser Tyr Val Pro Gln Glu Ala Ile		
275	280	285
Ser Ser Ala Ile Gln Leu Leu Glu Asp Pro Leu Gln Arg Glu Phe Ile		
290	295	300
Gln Lys Cys Leu Gln Ser Glu Pro Ala Arg Arg Pro Thr Ala Arg Glu		
305	310	315 320
Leu Leu Phe His Pro Ala Leu Phe Glu Val Pro Ser Leu Lys Leu Leu		
325	330	335
Ala Ala His Cys Ile Val Gly His Gln His Met Ile Pro Glu Asn Ala		
340	345	350
Leu Glu Glu Ile Thr Lys Asn Met Asp Thr Ser Ala Val Leu Ala Glu		
355	360	365
Ile Pro Ala Gly Pro Gly Arg Glu Pro Val Gln Thr Leu Tyr Ser Gln		
370	375	380
Ser Pro Ala Leu Glu Leu Asp Lys Phe Leu Glu Asp Val Arg Asn Gly		
385	390	395 400
Ile Tyr Pro Leu Thr Ala Phe Gly Leu Pro Arg Pro Gln Gln Pro Gln		
405	410	415
Gln Glu Glu Val Thr Ser Pro Val Val Pro Pro Ser Val Lys Thr Pro		
420	425	430
Thr Pro Glu Pro Ala Glu Val Glu Thr Arg Lys Val Val Leu Met Gln		
435	440	445
Cys Asn Ile Glu Ser Val Glu Glu Gly Val Lys His His Leu Thr Leu		
450	455	460
Leu Leu Lys Leu Glu Asp Lys Leu Asn Arg His Leu Ser Cys Asp Leu		
465	470	475 480
Met Pro Asn Glu Asn Ile Pro Glu Leu Ala Ala Glu Leu Val Gln Leu		

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485	490	495
Gly Phe Ile Ser Glu Ala Asp Gln Ser Arg Leu Thr Ser Leu Leu Glu		
500	505	510
Glu Thr Leu Asn Lys Phe Asn Phe Ala Arg Asn Ser Thr Leu Asn Ser		
515	520	525
Ala Ala Val Thr Val Ser Ser		
530	535	

## (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 39 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCACAGTCGA CCAAGCCGGA GTCGCAGAG

39

## (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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GCACAAAGCT TGCCAGGAGG GGTCTGGCTG

30

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCACAACCAT GGCCAAGCCG GAGTCGCAGG AG

32

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCACAAGATC TCCAGGAGGG GTCTGGCTG

29

## (2) INFORMATION FOR SEQ ID NO:15:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Lys Pro Glu Ser Gln Glu Ala Ala Pro Leu Ser Gly Pro Cys

5

10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

29



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## (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 50 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCACATCTAG ATCAGTGGTG GTGGTGGTGG TGGACCGGCC CCAGGAGTGG

50

## (2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCACAGCGGC CGCGAGGCCA TGGGCGCGCG C

31

## (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 52 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCACAGCGGC CGCTCAGTGG TGGTGGTGGT GGTGCCAGGA GGGGTCTGGC TG

52

## (2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTGACTTCCA TGCCATCCTT

20

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GCTCAGACT CCAATCTGAT

20

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 5 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Arg Ile Val Gly Gly  
5

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 959 base pairs
  - (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 2..856

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

C GAC CTA TTG TCA GGG CCC TGC GGT CAC AGG ACC ATC CCT TCC CGT	46
Asp Leu Leu Ser Gly Pro Cys Gly His Arg Thr Ile Pro Ser Arg	
1 5 10 15	
ATA GTG GGT GGC GAT GAT GCT GAG CTT GGC CGC TGG CCG TGG CAA GGG	94
Ile Val Gly Gly Asp Asp Ala Glu Leu Gly Arg Trp Pro Trp Gln Gly	
20 25 30	
AGC CTG CGT GTA TGG GGC AAC CAC TTA TGT GGC GCA ACC TTG CTC AAC	142
Ser Leu Arg Val Trp Gly Asn His Leu Cys Gly Ala Thr Leu Leu Asn	
35 40 45	
CGC CGC TGG GTG CTT ACA GCT GCC CAC TGC TTC CAA AAG GAT AAC GAT	190
Arg Arg Trp Val Leu Thr Ala Ala His Cys Phe Gln Lys Asp Asn Asp	
50 55 60	
CCT TTT GAC TGG ACA GTC CAG TTT GGT GAG CTG ACT TCC AGG CCA TCT	238
Pro Phe Asp Trp Thr Val Gln Phe Gly Glu Leu Thr Ser Arg Pro Ser	
65 70 75	
CTC TGG AAC CTA CAG GCC TAT TCC AAC CGT TAC CAA ATA GAA GAT ATT	286
Leu Trp Asn Leu Gln Ala Tyr Ser Asn Arg Tyr Gln Ile Glu Asp Ile	
80 85 90 95	
TTC CTG AGC CCC AAG TAC TCG GAG CAG TAT CCC AAT GAC ATA GCC CTG	334
Phe Leu Ser Pro Lys Tyr Ser Glu Gln Tyr Pro Asn Asp Ile Ala Leu	
100 105 110	
CTG AAG CTG TCA TCT CCA GTC ACC TAC AAT AAC TTC ATC CAG CCC ATC	382
Leu Lys Leu Ser Ser Pro Val Thr Tyr Asn Asn Phe Ile Gln Pro Ile	
115 120 125	

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TGC CTC CTG AAC TCC ACG TAC AAG TTT GAG AAC CGA ACT GAC TGC TGG	430
Cys Leu Leu Asn Ser Thr Tyr Lys Phe Glu Asn Arg Thr Asp Cys Trp	
130 135 140	
GTG ACC GGC TGG GGG GCT ATT GGA GAA GAT GAG AGT CTG CCA TCT CCC	478
Val Thr Gly Trp Gly Ala Ile Gly Glu Asp Glu Ser Leu Pro Ser Pro	
145 150 155	
AAC ACT CTC CAG GAA GTG CAG GTA GCT ATT ATC AAC AAC AGC ATG TGT	526
Asn Thr Leu Gln Glu Val Gln Val Ala Ile Ile Asn Asn Ser Met Cys	
160 165 170 175	
AAC CAT ATG TAC AAA AAG CCA GAC TTC CGC ACG AAC ATC TGG GGA GAC	574
Asn His Met Tyr Lys Lys Pro Asp Phe Arg Thr Asn Ile Trp Gly Asp	
180 185 190	
ATG GTT TGC GCT GGC ACT CCT GAA GGT GGC AAG GAT GCC TGC TTT GGT	622
Met Val Cys Ala Gly Thr Pro Glu Gly Gly Lys Asp Ala Cys Phe Gly	
195 200 205	
GAC TCG GGA GGA CCC TTG GCC TGC GAC CAG GAT ACG GTG TGG TAT CAG	670
Asp Ser Gly Gly Pro Leu Ala Cys Asp Gln Asp Thr Val Trp Tyr Gln	
210 215 220	
GTT GGA GTT GTG AGC TGG GGA ATA GGC TGT GGT CGC CCC AAT CGC CCT	718
Val Gly Val Val Ser Trp Gly Ile Gly Cys Gly Arg Pro Asn Arg Pro	
225 230 235	
GGA GTC TAT ACC AAC ATC AGT CAT CAC TAC AAC TGG ATC CAG TCA ACC	766
Gly Val Tyr Thr Asn Ile Ser His His Tyr Asn Trp Ile Gln Ser Thr	
240 245 250 255	
ATG ATC CGC AAT GGG CTG CTC AGG CCT GAC CCA GTC CCC TTG CTA CTG	814
Met Ile Arg Asn Gly Leu Leu Arg Pro Asp Pro Val Pro Leu Leu Leu	
260 265 270	
TTT CTT ACT CTG GCC TGG GCT TCC TCT TTG CTG AGG CCT GCC	856
Phe Leu Thr Leu Ala Trp Ala Ser Ser Leu Leu Arg Pro Ala	
275 280 285	
TGAGCCCA CA CGTG TACGTC ACACCTGTGA GGTCAGGGTG TGTCTCTTTT GTATCTTGCT	916
TGCTAATAAA CCTGTTAATA TTTAAAAAAA AAAAAAAAAA AAA	959

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## (2) INFORMATION FOR SEQ ID NO:26:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 285 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Asp Leu Leu Ser Gly Pro Cys Gly His Arg Thr Ile Pro Ser Arg Ile  
1 5 10 15

Val Gly Gly Asp Asp Ala Glu Leu Gly Arg Trp Pro Trp Gln Gly Ser  
20 25 30

Leu Arg Val Trp Gly Asn His Leu Cys Gly Ala Thr Leu Leu Asn Arg  
35 40 45

Arg Trp Val Leu Thr Ala Ala His Cys Phe Gln Lys Asp Asn Asp Pro  
50 55 60

Phe Asp Trp Thr Val Gln Phe Gly Glu Leu Thr Ser Arg Pro Ser Leu  
65 70 75 80

Trp Asn Leu Gln Ala Tyr Ser Asn Arg Tyr Gln Ile Glu Asp Ile Phe  
85 90 95

Leu Ser Pro Lys Tyr Ser Glu Gln Tyr Pro Asn Asp Ile Ala Leu Leu  
100 105 110

Lys Leu Ser Ser Pro Val Thr Tyr Asn Asn Phe Ile Gln Pro Ile Cys  
115 120 125

Leu Leu Asn Ser Thr Tyr Lys Phe Glu Asn Arg Thr Asp Cys Trp Val  
130 135 140

Thr Gly Trp Gly Ala Ile Gly Glu Asp Glu Ser Leu Pro Ser Pro Asn  
145 150 155 160

Thr Leu Gln Glu Val Gln Val Ala Ile Ile Asn Asn Ser Met Cys Asn  
165 170 175

His Met Tyr Lys Lys Pro Asp Phe Arg Thr Asn Ile Trp Gly Asp Met

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180	185	190
Val Cys Ala Gly Thr Pro Glu Gly Gly Lys Asp Ala Cys Phe Gly Asp		
195	200	205
Ser Gly Gly Pro Leu Ala Cys Asp Gln Asp Thr Val Trp Tyr Gln Val		
210	215	220
Gly Val Val Ser Trp Gly Ile Gly Cys Gly Arg Pro Asn Arg Pro Gly		
225	230	235
Val Tyr Thr Asn Ile Ser His His Tyr Asn Trp Ile Gln Ser Thr Met		
245	250	255
Ile Arg Asn Gly Leu Leu Arg Pro Asp Pro Val Pro Leu Leu Leu Phe		
260	265	270
Leu Thr Leu Ala Trp Ala Ser Ser Leu Leu Arg Pro Ala		
275	280	285

## (2) INFORMATION FOR SEQ ID NO:27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3866 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AGTGAGTCTC CTGCCTCAGC CTCCCAAGTA GCTGGGACTT CAGGTGTGTG CCACCATCCT	60
CAGCTAATTT TTTTTTTTTT TTTTTTTTTT AGAAGGAGTC TTGCTCTGTC GCCCAGGCTG	120
GAGTGCAGTG GCGCGATCTT CCAGGCCCCA CCGGGCCCTC AGGAAGGCCT TGCCTACCTG	180
CTTTAAGGGG ACTCCTGGCT CAGGGCCAGG CCCCTGGTGC TGGAGGAGGT GGTGGGTGGA	240
GGGCAGGGGG CACCAAGCGG GCAGCCAGGA CCCCCGGGCT GCAGACAAGA AAAGGACTGT	300

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GGGGTCCACC GGGTCTGGGC CACATCAAGG AATGTGGTTG AAGACCCGCC CTTAGGAGCT	360
GAAAGCCAGG GCGCTACCAG GCCTGAGAGG CCCCAAACAG CCCTTGGGCC TGGTTTGGGA	420
GGATTAAGCT GGAGCTCCCA ACCCGCCCTG CCCCAGGGG GCGACCCCGG GCCCGGCGCG	480
AGAGGAGGCA GAGGGGGCGT CAGGCCGCGG GAGAGGAGGC CATGGGCGCG CGCGGGGCGC	540
TGCTGCTGGC GCTGCTGCTG GCTCGGGCTG GACTCAGGAA GCCGGGTGAG CTCGGGGCGC	600
TGCTGGCGGG ATGGGGAGGC GGGGGAGCGG TGGGGAGGAC GGGAGGTGGA GGCCGCGGGG	660
AGTCACTTCT TGTCTCCCGC AGAGTCGCAG GAGGCGGCGC CGTTATCAGG TAGGGCGCCC	720
AGGACGCGCG ATTCTTGCCA GGGCCGTTGG GCCGAGGTGG ACGGGGGGCG GTGAGGGGGT	780
AGAGGGGGGC CTTTACTGCT CTCTCGCCCC CGCCCCCGG ATCGAGAACT CTGTTGGCGT	840
GGAAAGTAAC TAACGGACGC TGGAGGGGGA TGGGCGGGCC CTGCAGAGCA CGTGGGAGGA	900
TCTCCAGTGT CACCTACTTC CTGCTGCACA CACGCGAGGG GACCCTGGGT GGGCAAAAAC	960
GTGCTTTCCC GGACGGGGTT GAAGGGGAGA AAGGGAGAGG TCGGGCTTGG GGGGCTGCCT	1020
CCCGCGGCTC AGCAGTTCCT CTGACCATCC GAGGACCATG CGGCCGACGG GTCATCACGT	1080
CGCGCATCGT GGGTGGAGAG GACGCCGAAC TCGGGCGTTG GCCGTGGCAG GGGAGCCTGC	1140
GCCTGTGGGA TTCCCACGTA TGCCGAGTGA GCCTGCTCAG CCACCECTGG GCACTCACGG	1200
CGGCGCACTG CTTTGAAACG TGAGTGGGGG TGCGAACGGA GGGGTGCGGG GACGGGCAGG	1260
AACAGGGCTG GAGGGAGTGC CACCGAACTT TACCTCTGGT CTGATGCCAG ACTTGGGCGT	1320
GAAAGTTGTG CGTGGATGCG GCCTGGTGTT CTCCTGAGCC CCAGGCTGTG CTGCAGCCGG	1380
TTACACCCAC TCCAGTTCCT TTTGGGTCTC CTGGAGGGAA CCCTGTTCAG GTTATTCCAG	1440
AATGTTCTTC CAGAACATTT CCACACACTT TTGGGTATTC TCTCCCTTTT TCTTTCAACC	1500
CAAAGTTCAC CACTGACCAT CCCACCCTCA TCCCCCTCC TGGTGGACGG TCGGTACAG	1560
TGTGGGGCAC TGAGCCAAGG CCAGCACCCC CGGGCCGCTG TGTGGACTCC ATCCTGCCAA	1620
TCCCACATTG GCGTGGTGCA TCTCCCCATT CCTCCTTGGG CTGCATGGGG GTGCCCCCTG	1680

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AGGCCTTGGC TCAATGCAAG GCTCCTTGGG ACAGCTCTGG GAGGTGACAA GACCCACCC	1740
TTCTGCTGCA GGAGCAGGTC CTAGGACTTT GGTGTGGTC TGTCTGGGCT CCTTCATTC	1800
TGCAGGGGAC CCTGGGTGTT AGCAAGTAGC AGCAACACCA CAGTTTCCCC TCCTGCACTG	1860
GACCCAGTT GTGCTCAGGT AGCCAGCCCT CCATCCAGGG CCCCTGACTG CTCTCTCTC	1920
TTCTGCCAGC TATAGTGACC TTAGTGATCC CTCCGGGTGG ATGGTCCAGT TTGGCCAGCT	1980
GACTTCCATG CCATCCTTCT GGAGCCTGCA GGCCTACTAC ACCCGTTACT TCGTATCGAA	2040
TATCTATCTG AGCCCTCGCT ACCTGGGGAA TTCACCCTAT GACATTGCCT TGGTGAAGCT	2100
GTCTGCACCT GTCACCTACA CTAAACACAT CCAGCCCATC TGTCTCCAGG CCTCCACATT	2160
TGAGTTTGAG AACCGGACAG ACTGCTGGGT GACTGGCTGG GGGTACATCA AAGAGGATGA	2220
GGGTGAGGCT GGGGACAGGC GGGTCAGGA GGAAGTGTCT TTGTTACCT GTTCCCCTGC	2280
ATAGGCACAA TAGCCCCCTG CTTGGTCTGG GGGTGCAGGC TATGCCCCTC TTGCTTGCA	2340
TCTCTCTCA CCTGCCAGG CAGGGACCAA ACACCCAGTT CTCTCCCTTC CAGGGGCTGT	2400
GGGGGCCAGA AGGAGAGTGT GAGAGGGAGG CCAGTTTGGC GCAAGCCTGT GGGTGGTGCG	2460
GTGGTGGAGG GGTCTGGAG GGCTTGGCGA CATAAACCTC ATACTTGGAT TTATTCCTGC	2520
ATCTTTCCAC CTCCCCAGT GCTACCAAT GCCCAGGCA TCACCAGTT GCCCCTTCCC	2580
CCAAGGTCTG GCTTTGGATG CTTATGTGAA CACCGTTTTA AGTTGCCTTG GCCCCTTCCT	2640
CGGTTCTTT TTGGCTGAGG AATCTCTCCA TGGCTGCAGG CAGGGCCATT GTTGCCATTC	2700
TACAGATAGG GAAAGTGCGG CTGGGGGAGC TCTGACAGCT GTCCCTCCCC GGGGCCTTCT	2760
GTGATGCTGC TGAGGGCCTC TGTGTGCTG GGGTCTGGGT TGGAGCTGGG GGTAATGGAG	2820
ATGAACCTGC CAGGCACAGT GGGTGCCCCA GGGCCCCAC CCCCAGCC TATGCCATCC	2880
CTCCATAGAG GGGCCTCAGG TTGCTGTCTC TCTCCTTCCC ACTATCGTCC GCACAGCACT	2940
GCCATCTCCC CACACCCTCC AGGAAGTTCA GGTCGCCATC ATAAACAACT CTATGTGCAA	3000
CCACCTCTTC CTCAAGTACA GTTTCGCAA GGACATCTTT GGAGACATGG TTTGTGCTGG	3060



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CAATGCCCAA GGCGGGAAGG ATGCCTGCTT CGTGAGTGTC CTTGCCACCA CTCCCAGCCC	3120
AGGAAAGCAT CCTGTGTCCC TGTGCCTTAT TTGACCCTCA TGCCAACCCC GGGAGGTGGA	3180
GACTGTTGCC CCACTCTGCA GATGCAGAAA CGGAGGCTTG GCTGCTGCCA GGGGGAGGAG	3240
GAGGATGTGC ACCCAGTCTA CCCAGCCCCA TAGCCCTTCC CACTCTCAGC CCCTCCCCTG	3300
CCCCACTCAC TCTGCCCCAG GCTGACCTCA GCCCCGCTGC TCCCCAGGGT GACTCAGGTG	3360
GACCCTTGGC CTGTAACAAG AATGGACTGT GGTATCAGAT TGGAGTCGTG AGCTGGGGAG	3420
TGGGCTGTGG TCGGCCCAAT CGGCCCCGTG TCTACACCAA TATCAGCCAC CACTTTGAGT	3480
GGATCCAGAA GCTGATGGCC CAGAGTGGCA TGTCCCAGCC AGACCCCTCC TGGCCGCTAC	3540
TCTTTTTCCC TCTTCTCTGG GCTCTCCCAC TCCTGGGGCC GGTCTGAGCC TACCTGAGCC	3600
CATGCAGCCT GGGGCCACTG CCAAGTCAGG CCCTGGTTCT CTTCTGTCTT GTTTGGTAAT	3660
AAACACATTC CAGTTGATGC CTTGCAGGGC ATTCTTCAAA AGCAGTGGCT TCATGGACAG	3720
CTCATTCTCT CTTGTGCAGA CAGCCTGTCT GTGCCCCTGG CTCACACCCA CATCTGTTCT	3780
GCACCATAGA ACCATCTGGT TATTTCGATC AGAAAGAGAA TTGTGTGTTG CCCAGGCTGG	3840
TCTTGAACGC CTAGGGTGTC TCGATC	3866

## (2) INFORMATION FOR SEQ ID NO:28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1165 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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GGGGTCCACC GGGTCTGGGC CACATCAAGG AATGTGGTTG AAGACCCGCC CTTAGGAGCT	360
GAAAGCCAGG GCGCTACCAG GCCTGAGAGG CCCCAAACAG CCCTTGGGCC TGGTTTGGGA	420
GGATTAAGCT GGAGCTCCCA ACCCGCCCTG CCCCCAGGGG GCGACCCCGG GCCCGGCGCG	480
AGAGGAGGCA GAGGGGGCGT CAGGCCGCGG GAGAGGAGGC CATGGGCGCG CGCGGGGCGC	540
TGCTGCTGGC GCTGCTGCTG GCTCGGGCTG GACTCAGGAA GCCGGGTGAG CTCGGGGCGC	600
TGCTGGCGGG ATGGGGAGGC GGGGGAGCGG TGGGGAGGAC GGGAGGTGGA GGCCGCGGGG	660
AGTCACTTCT TGTCTCCCGC AGAGTCGCAG GAGGCGGCGC CGTTATCAGG TAGGGCGCCC	720
AGGACGCGCG ATTCCTGCCA GGGCCGTTGG GCCGAGGTGG ACGGGGGGCG GTGAGGGGGT	780
AGAGGGGGGC CTTTACTGCT CTCTCGCCCC CGCCCCCGGG ATCGAGAACT CTGTTGGCGT	840
GGAAAGTAAC TAACGGACGC TGGAGGGGGA TGGGCGGGCC CTGCAGAGCA CGTGGGAGGA	900
TCTCCAGTGT CACCTACTTC CTGCTGCACA CACGCGAGGG GACCCTGGGT GGGCAAAAAC	960
GTGCTTTCCC GGACGGGGTT GAAGGGGAGA AAGGGAGAGG TCGGGCTTGG GGGGCTGCCT	1020
CCC GCGGCTC AGCAGTTCCT CTGACCATCC GAGGACCATG CGGCCGACGG GTCATCACGT	1080
CGCGCATCGT GGGTGGAGAG GACCCGAAC TCGGGCGTTG GCCGTGGCAG GGGAGCCTGC	1140
GCCTGTGGGA TTCCACGTA TGCCGAGTGA GCCTGCTCAG CCACCGCTGG GCACTCACGG	1200
CGGCGCACTG CTTTGAAACG TGAGTGGGGG TGCGAACGGA GGGGTGCGGG GACGGGCAGG	1260
AACAGGGCTG GAGGGAGTGC CACCGAACTT TACCTCTGGT CTGATGCCAG ACTTGGGCGT	1320
GAAAGTTGTG CGTGGATGCG GCCTGGTGTT CTCCTGAGCC CCAGGCTGTG CTGCAGCCGG	1380
TTACACCCAC TCCAGTTCCC TTTGGGTCTC CTGGAGGGAA CCCTGTTCAG GTTATTCCAG	1440
AATGTTCTTC CAGAACATTT CCACACACTT TTGGGTATTC TCTCCCTTTT TCTTTCAACC	1500
CAAAGTTCAC CACTGACCAT CCCACCCTCA TCCCCCTCC TGGTGGACGG TGCGGTACAG	1560
TGTGGGGCAC TGAGCCAAGG CCAGCACCCC CGGGCCGCTG TGTGGACTCC ATCCTGCCAA	1620
TCCCACATTG GCGTGGTGCA TCTCCCCATT CCTCCTTGGG CTGCATGGGG GTGCCCCCTG	1680

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AGGCCTTGGC TCAATGCAAG GCTCCTTGGG ACAGCTCTGG GAGGTGACAA GACCCACCCC	1740
TTCTGCTGCA GGAGCAGGTC CTAGGACTTT GGTGTGGTTC TGTCTGGGCT CCTTCATTTTC	1800
TGCAGGGGAC CCTGGGTGTT AGCAAGTAGC AGCAACACCA CAGTTTCCCC TCCTGCACTG	1860
GACCCAGTT GTGCTCAGGT AGCCAGCCCT CCATCCAGGG CCCCTGACTG CTCTCTTCTC	1920
TTCTGCCAGC TATAGTGACC TTAGTGATCC CTCCGGGTGG ATGGTCCAGT TTGGCCAGCT	1980
GACTTCCATG CCATCCTTCT GGAGCCTGCA GGCCTACTAC ACCCGTTACT TCGTATCGAA	2040
TATCTATCTG AGCCCTCGCT ACCTGGGGAA TTCACCTAT GACATTGCCT TGGTGAAGCT	2100
GTCTGCACCT GTCACCTACA CTAAACACAT CCAGCCCATC TGTCTCCAGG CCTCCACATT	2160
TGAGTTTGAG AACCGGACAG ACTGCTGGGT GACTGGCTGG GGGTACATCA AAGAGGATGA	2220
GGGTGAGGCT GGGGACAGGC GGGTCAGGGA GGAACGTCTT TTGTTACCT GTTCCCCTGC	2280
ATAGGCACAA TAGCCCCCTG CTTGGTCTGG GGGTGCAGGC TATGCCCCCTC TTGCTTGCAG	2340
TCTCTCCTCA CCTGCCAGGG CAGGGACCAA ACACCCAGTT CTCTCCCTTC CAGGGGCTGT	2400
GGGGGCCAGA AGGAGAGTGT GAGAGGGAGG CCAGTTTGGC GCAAGCCTGT GGGTGGTGCG	2460
GTGGTGGAGG GGTCTTGAG GGCTTGGCGA CATAAACCTC ATACTTGGAT TTATTCCTGC	2520
ATCTTTCAC CTCCCCAGT GCTCACCAAT GCCCCAGGCA TCACCAGCTT GCCCCTTCCC	2580
CCAAGGTCTG GCTTTGGATG CTTATGTGAA CACCGTTTTA AGTTGCCTTG GCCCCCTCCT	2640
CGGTTCCTTT TTGGCTGAGG AATCTCTCCA TGGCTGCAGG CAGGGCCATT GTTGCCATTC	2700
TACAGATAGG GAAAGTGCGG CTGGGGGAGC TCTGACAGCT GTCCCTCCCC GGGGCCTTCT	2760
GTGATGCTGC TGAGGGCCTC TGTGTGCTG GGGTCTGGGT TGGAGCTGGG GGTAATGGAG	2820
ATGAACCTGC CAGGCACAGT GGGTGCCCCA GGGCCCCAC CCCCAGCC TATGCCATCC	2880
CTCCATAGAG GGGCCTCAGG TTGCTGTCTC TCTCCTTCCC ACTATCGTCC GCACAGCACT	2940
GCCATCTCCC CACACCCTCC AGGAAGTTCA GGTGCGCATC ATAAACAACT CTATGTGCAA	3000
CCACCTCTTC CTCAAGTACA GTTCCGCAA GGACATCTTT GGAGACATGG TTTGTGCTGG	3060